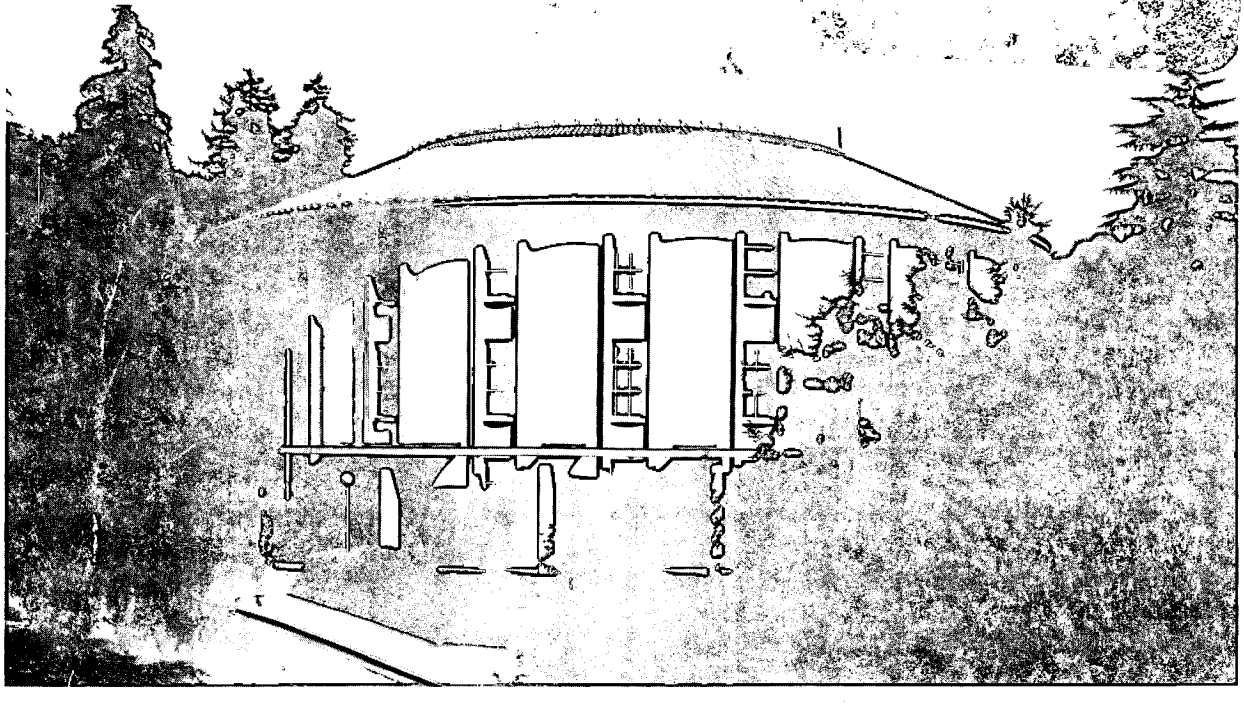
Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

CHEMICAL BIODYNAMICS DIVISION

Chemical Biodynamics Division Annual Report
October 1, 1986–September 30, 1987

September 1987



Prepared for the U.S. Department of Energy under Contract DE-AC03-76SF00098

DISCLAIMER

CHEMICAL BIODYNAMICS DIVISION

Annual Report

October 1, 1986 – September 30, 1987

Lawrence Berkeley Laboratory

University of California
Berkeley, CA 94720

Prepared for the U.S. Department of Energy
Contract No. DE-AC03-76SF00098

ANNUAL REPORT, CHEMICAL BIODYNAMICS DIVISION

October 1, 1986 - September 30, 1987

TABLE OF CONTENTS

This report was prepared by James C. Bartholomew with the expert assistance of Gloria Goldberg, Beth Klingel, Gary Smith, and Lois Soulé.

INTRODUCTION

The Laboratory of Chemical Biodynamics (LCB) was established in 1945 as a Division of the Lawrence Berkeley Laboratory to conduct basic research on the dynamics of living cells and on the interaction of radiant energy with organic matter. The Division has made rich contributions to our understanding of the molecular mechanisms of photosynthesis and of the effects of environmental pollutants on plant and animal cells. Much of its funding is provided by the U.S. Department of Energy and the National Institutes of Health through the Lawrence Berkeley Laboratory. LCB is also an Organized Research Unit (ORU) of the University of California and additional reseach support comes to the ORU from the National Science Foundation, the National Institute of Health, and from private and industrial grants-in-aid. Chemical Biodynamics draws its M.S. and Ph.D. candidates from over thirteen departments and groups of the University, with the single largest component being in chemistry. The Division attracts research scientists from all over the world; during the current year 11 foreign postdoctoral and visiting faculty personnel from seven countries have conducted research in the Chemical Biodynamics Laboratory.

Four broad aims guide the Division:

- to maintain the strong interdisciplinary character of the laboratory.
- to build upon the expertise of the existing laboratory staff and to exploit the excellent array of facilities in LCB.
- to develop coherent and contemporary research themes that are fundamental in character, forward looking in their broad direction, and that optimize the likelihood of synergistic interaction across the disciplines represented in the laboratory.
- to strengthen collaborative ties to faculty research scientists in the biological sciences.

Two research themes are being developed that are consistent with these aims. Both are directed at fundamental areas of knowledge essential to the most effective utilization of solar energy. The first theme, **Photon Conversion**, pertains to the fundamental aspects of the interaction of light with matter; the absorption, migration, relaxation, transformation, and storage of energy derived from photons. It will extend from photobiology, already a strong area within the laboratory, through photochemistry to photophysics.

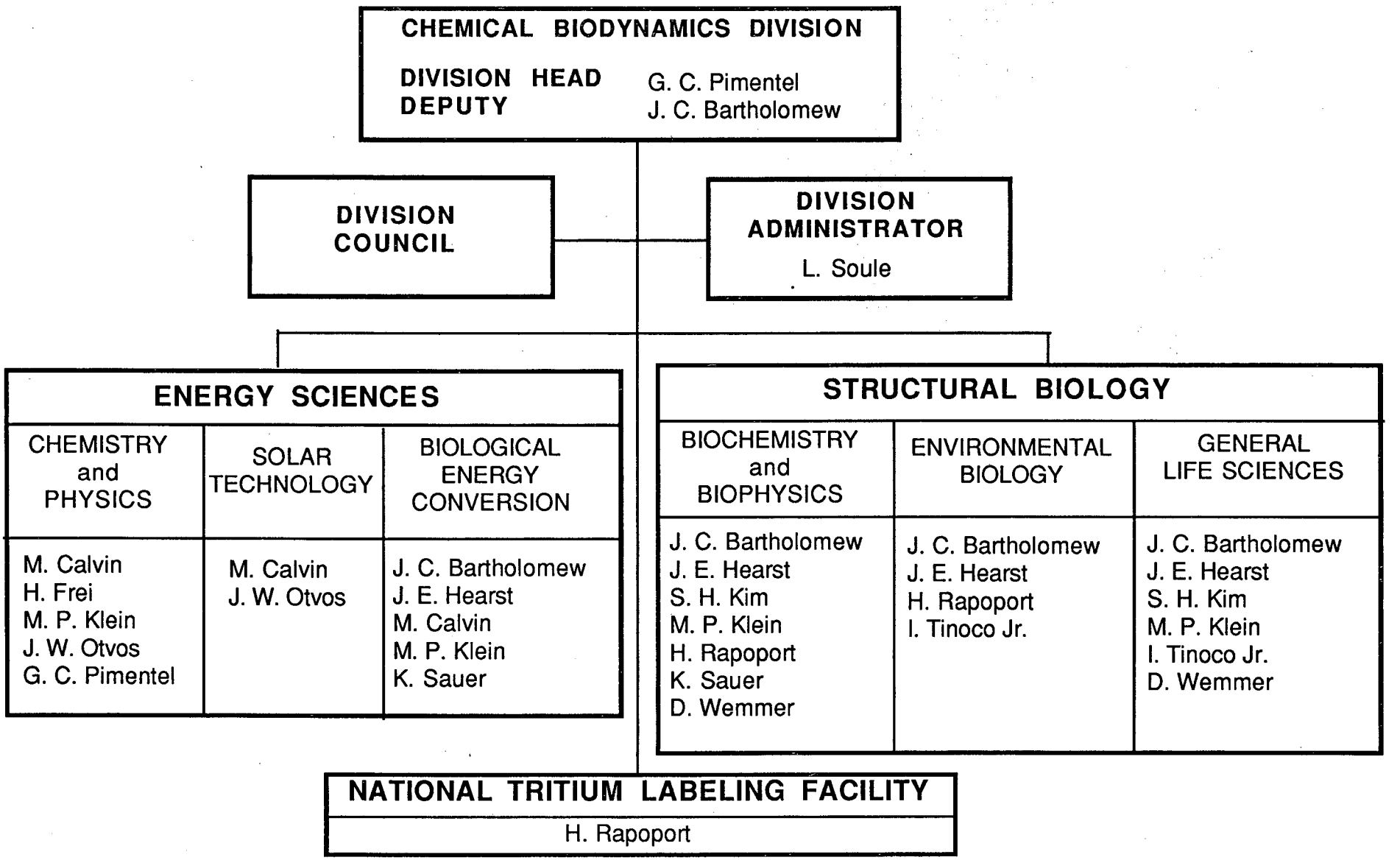
The second theme, **Genetics of Photosynthesis**, is the application of recombinant DNA techniques to photosynthetic organisms. The staff's experience in molecular biology, plant cell culture, and the functional mechanisms of the chloroplast are being exploited as we investigate and attempt to control the genetic material from which the photosynthetic apparatus arises.

The new initiatives sponsored by me during the past year are the **Human Genome Center** and the **Center for Structural Biology**.

John E. Hearst, Acting Associate Director
Chemical Biodynamics Division

LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA

CHEMICAL BIODYNAMICS DIVISION



OVERVIEW OF RESEARCH

The Laboratory of Chemical Biodynamics continues to base its research plan on the importance of its role in the application of sophisticated chemical sciences to problems relevant to the mission of the Department of Energy. Our Laboratory is in the unique position that it is staffed mainly by investigators trained in chemistry with an interest in applying these skills to both biological and energy science problems. Our mission is to carry out research that takes advantage of our unique skills, as well as to train young investigators in the fields we so strongly represent.

The research in the Laboratory of Chemical Biodynamics is almost entirely fundamental research. The biological research component is strongly dominated by a long term interest in two main themes which make up our Structural Biology Program. The first interest has to do with understanding the molecular dynamics of photosynthesis. The Laboratory's investigators are studying the various components that make up the photosynthetic reaction center complexes in many different organisms. This work not only involves understanding the kinetics of energy transfer and storage in plants, but also includes studies to work out how photosynthetic cells regulate the expression of genes encoding the photosynthetic apparatus. The second biological theme is a series of investigations into the relationship between structure and function in nucleic acids. Our basic mission in this program is to couple our chemical and biophysical expertise to understand how not only the primary structure of nucleic acids, but also higher levels of structure including interactions with proteins and other nucleic acids regulate the functional activity of genes.

In the chemical sciences work in the Laboratory, our investigators are increasing our understanding of the fundamental chemistry of electronically excited molecules, a critical dimension of every photosynthetic energy storage process. We are developing approaches not only toward the utilization of sophisticated chemistry to store photon energy, but also to develop systems that can emulate the photosynthetic apparatus in the trapping and transfer of photosynthetic energy.

KCO3 – CHEMICAL SCIENCES

This program encompasses research directed at a fundamental understanding of electronically excited molecules with special attention to features that relate to the storage of photon energy in the form of high free energy chemical bonds.

- One project focuses on the manganese catalytic function in the oxidation aspect of artificial photosynthesis, the photo-induced reduction of CO_2 into organic products potentially useful as fuels, and charged colloid or polyelectrolyte interfaces for increasing quantum efficiency in photosynthetic processes based on electon transfer.

- A second project spotlights the special chemistry of electronically excited molecules and atoms using infrared spectroscopy as a diagnostic tool and tuned laser excitation to map electronic reaction hypersurfaces, both for unimolecular (photochromic) and biomolecular reactions.

- The third project in this program investigates the indefinite storage of long-lived electronically excited molecules including those that can be initially prepared with near infrared photons, the spectral region in which most of the solar energy is found.

1. THE CHEMISTRY OF ELECTRONICALLY EXCITED MOLECULES – Professor G. C. Pimentel

This research is directed toward fundamental understanding of the special chemistry of electronically excited molecules, which is involved in every photosynthetic photon energy storage process. An electronically excited molecule differs from the ground state in orbital occupancy, charge distribution, molecular structure, and chemical reactivities. These differences are the key to photon energy storage.

Infrared spectroscopy coupled with matrix isolation provides a powerful diagnostic technique. Absorption features are sharp and informative about molecular structures. With a tunable laser photolysis source, we are attempting to map electronic hypersurfaces. Both unimolecular (photochromic) and bimolecular reactions are under study. To increase our knowledge of matrix-induced surface crossing, we are investigating fluorescence and phosphorescence as well.

Fluorescence and Phosphorescence of Dimethyl Amino Benzonitrile

The compound dimethylamino benzonitrile (DMABN) has received a great deal of attention because in room temperature solutions it displays two fluorescent radiative relaxation paths. These two paths are strongly solvent–and temperature-dependent. The strong solvent dependence is attributed to a very large charge separation (molecular dipole moment) in the electronically excited state. This excited state is, of course, stabilized in polar solvents which affects the dual fluorescence.

To add new information and elucidate further this interesting behavior, we have investigated the fluorescence and phosphorescence of DMABN suspended in various matrices at 10°K. Both inert gas and polar matrices have been investigated. With the three inert gas matrices Ar, Kr, and Xe, both fluorescence and phosphorescence were observed. As the spin-orbit coupling constant of the inert gas increased (i.e., in the sequence Ar to Kr to Xe), the amount of fluorescence decreased and the amount of phosphorescence increased. At the same time, the sum of fluorescence plus phosphorescence increased. This shows that the most important effect of the matrix environment is to increase singlet-triplet surface crossing.

This is the first clear-cut demonstration of phosphorescence from DMABN and our spectra display interpretable vibrational fine structure that gives information about the ground state. When a polar matrix, such as ammonia (μ = 1.47D) or hydrogen bromide (μ = 0.79D) is used, the vibrational fine structure is lost but, surprisingly, the zero-zero frequencies of both the singlet and the triplet transitions are unaffected. The significance of this striking difference from the polar solvent affects observed in solutions is under study.

Column 2B Metal-Olefin Reactions

We are continuing our study of the matrix reactions between Hg, Cd, and Zn atoms excited to the ^3P state with halogenated olefins. In progress so far, direct photolytic excitation of

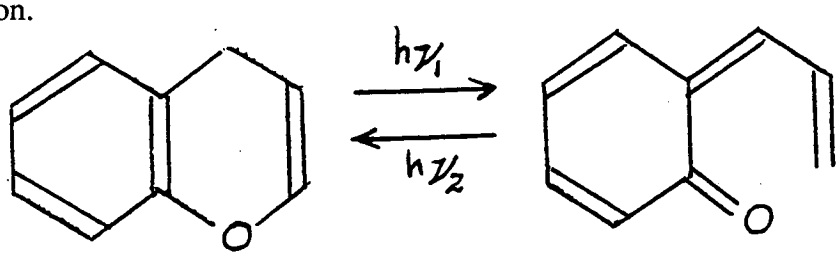
dichloroethenes results in HCl elimination whereas when Hg is added and selectively excited to the ^3P state, the reaction products change. The HCl elimination product is not observed and, instead Cl_2 elimination takes place. In addition, mercury insertion into the C–Cl bond occurs, the first demonstration of this reaction by photochemical means. We attribute the change in products to the change from chemistry on a singlet reaction surface to the different chemistry of a triplet reaction surface. To test this interpretation, we have carried out the direct photolysis experiment (in absence of mercury) in xenon matrix. Because of its strong spin-orbit coupling, xenon has been shown in our earlier work to facilitate transfer from singlet to triplet surfaces (or vice versa, of course). In agreement with our model, direct excitation of cis-1,2-dichloroethene in xenon at 10°K gave both HCl and Cl_2 elimination.

Emphasis is now being shifted toward Cd and Zn atoms because both singlet and triplet metal atom states are accessible with our tuned laser photolysis source. A Knudsen cell oven suited to controlled deposition of Cd and Zn atoms is under design and construction.

Electronically Induced Unimolecular Reactions: Photochromic Systems

We have investigated the photochromic phenylazirine ⟷ ylide system and, using IR matrix spectroscopy, established unequivocally the molecular structure of the ylide. With this starting point, we have investigated the photolytic interconversion as a function of wavelength, with the intent of searching for a double minimum potential surface in the electronically excited state. The experiment presented some ambiguities associated with overlapping absorptions of the azirine and the ylide. Nevertheless, the data provided no evidence for a threshold for interconversion that would be characteristic of a potential barrier in the excited state surface. Our tentative conclusion is that the photochromic interconversion occurs on a surface with a single potential minimum arising from surface crossing.

We are now investigating other possible photochromic systems that might involve a double minimum upper surface. The benzopyrans shown below, are typical of a number of molecules under consideration.



Electronically Induced Bimolecular Reactions

We have focussed attention on the photolysis of hydrogen halide-acetylene complexes in cryogenic matrices because the complex places the two potential reactants in close proximity and in known geometry. This permits us to investigate the photochemistry when the excited state is characteristic of the "supermolecule", the $HX \cdot C_2H_2$ complex, rather than of either of the individual reactant molecules.

The HBr-acetylene system has been well studied using broad band excitation. One possible reaction, the addition of HBr to give vinyl bromide must be considered, even though vinyl bromide does not accumulate as a product. Deposition of vinyl bromide with subsequent photolysis gives predominantly HBr elimination. In contrast, photolysis of HBr · C_2H_2 shows that DBr · C_2HD is formed, but also BrCCD + HD and BrCCH + D_2 are substantial products. These latter products plainly indicate that the main photolysis channel is not vinyl bromide formation followed by secondary photolysis. We attribute the hydrogen elimination to the photochemistry of the "supermolecule".

We have now extended this study to HI · acetylene, using tuned laser excitation. Using HI · C_2D_2, we can readily measure the relative amounts of hydrogen halide formation (through the growth of DI · C_2HD) and hydrogen formation (through the growth of C_2DI). There is plainly a wavelength dependence for the relative importance of these two reaction channels, over the range 222 to 304 nm. At short wavelengths, the favored reaction is exchange–reaction (1).

$$HI \cdot C_2D_2 \xrightarrow[222nm]{} DI \cdot C_2HD \qquad (1)$$

At longer wavelengths, hydrogen elimination occurs, reaction (2)

$$HI \cdot C_2D_2 \xrightarrow[304nm]{} HD \cdot C_2DI \qquad (2)$$

We are attempting to understand these trends in terms of the potential function of HI as perturbed by the nearby acetylene molecule, i.e., in terms of the potential function of the HI · C_2D_2 "supermolecules."

PHOTON CONVERSION THROUGH STORAGE OF METASTABLE MOLECULES - Dr. H. Frei

The purpose of this program is to search for and evaluate chemical systems which permit use and storage of near infrared photons, and allow to accomplish their efficient conversion into useful energy. The importance for exploring chemistry with these long wavelength quanta derives from the fact that photochemical reactions which can be initiated by these photons are very sparce despite the fact that more than half of the solar irradiance at ground level lies in the near infrared (wavelengths longer than 700 nm). Our work is aimed at contributing to this key problem in solar photochemistry in two ways. First, on a fundamental level, we are searching for low energy paths of bimolecular reactions that would allow us to initiate the chemistry with near infrared photons. Secondly, on a level directly aiming at photon storage and conversion, we are studing chemical systems which permit synthesis with near infrared photons, accomplish their storage, and offer a way for efficient conversion of the stored chemical energy into useful energy.

Chemical Storage of Singlet Oxygen

Endoperoxides of aromatic hydrocarbons like alkyl substituted naphthalenes are known to regenerate the parent hydrocarbon fragment and molecular oxygen upon thermal decomposition in the dark. Early experiments with singlet oxygen chemical traps led to the very interesting finding that most O_2 molecules are expelled in the $^1\Delta$ excited state, carrying one eV of electronic energy. This property, which has its origin in the conservation of spin along the decomposition path, makes these endoperoxides a form of chemically stored $O_2(^1\Delta)$; hence, they may permit storage of near infrared photon energy in an efficiently and easily retrievable form.

We have concentrated on the study of the elementary processes following the release of $O_2(^1\Delta)$ from two endoperoxides in room temperature solution, that of 1,4-dimethylnaphthalene (in aprotic solvents) and of 3,3′(1,4-naphthylidene) dipropionate endoperoxide (in aqueous solution). At the heart of the experiments is the direct observation of $O_2(^1\Delta)$ eliminated from these endoperoxides by its very weak $^1\Delta \rightarrow ^3\Sigma^-$ chemiluminescence at 1.3 micron, both upon thermolysis and upon excitation of the storage molecules to the S_2 excited state at 266 nm with 5 nsec laser pulses. Observation of near infrared chemiluminescence upon UV excitation gave the first direct evidence for elimination of $O_2(^1\Delta)$ from electronically excited endoperoxides, and allowed us to study the deactivation of the expelled singlet O_2 in real time.

The lifetime of $O_2(^1\Delta)$ expelled from the ionic endoperoxide in H_2O, D_2O and CH_3OH was found to be the same as observed upon photosensitization of singlet O_2 in these solvents (H_2O : $4\mu sec$; D_2O : $63\mu sec$; CH_3OH : $10\mu sec$). Hence, as anticipated, the decay of the photoeliminated $O_2(^1\Delta)$ is determined by solvent collisional deactivation.

Surprisingly, lifetimes substantially shorter than those reflecting mere relaxation by the solvent were measured for $O_2(^1\Delta)$ photoeliminated from 1,4-dimethylnaphthalene (DMN) endoperoxide in CH_3CN and CD_3CN. In order to elucidate the processes that dictate the decay kinetics of the expelled singlet oxygen, we have studied the laser pulse energy and concentration dependence of the DMN endoperoxide chemiluminescence decay. We found a sharp increase of the decay rate when increasing either laser pulse energy or endoperoxide concentration, which suggests that a photofragment of the endoperoxide, produced together with $O_2(^1\Delta)$, acts as an efficient singlet oxygen quencher. Logarithm of intensity versus time plots showed that this additional $O_2(^1\Delta)$ quenching process does not follow first order behavior, indicating that the quencher is either consumed upon encounter with $O_2(^1\Delta)$, or removed by some other chemical process during the lifespan of singlet oxygen.

We have identified two photofragments that quench the singlet O_2 expelled from the endoperoxide, O_2^- and $O_2(^1\Delta)$ itself. In a separate series of photosensitization experiments, we have determined a bimolecular rate constant of 3.10^7 l mole^{-1} sec^{-1} for the energy pooling process

$$O_2(^1\Delta_g) + O_2(^1\Delta_g) \rightarrow O_2(^1\Sigma_g^+) + O_2(^3\Sigma_g^-)$$

Despite this low value, it does contribute to the lifetime shortening of the photoexpelled $O_2(^1\Delta)$ at high singlet oxygen concentrations, in particular in solvents like CD_3CN which have a low

$O_2(^1\Delta)$ collisional deactivation rate.

The main cause of the lifetime shortening of the expelled $O_2(^1\Delta)$ are collisions with superoxide, known to quench singlet oxygen extremely efficiently

$$O_2(^1\Delta_g) + O_2^- \rightarrow O_2^- + O_2(^3\Sigma_g^-)$$

O_2^- may emerge, together with the dimethylnaphthalene radical cation, along an ionic endoperoxide fragmentation path. Diffusion controlled back electron transfer between the ions would lead to depletion of superoxide on the microsecond time scale, hence affect the $O_2(^1\Delta)$ decay kinetics in a nonexponential fashion.

We have solved the kinetic problem for simultaneous deactivation of the photoeliminated $O_2(^1\Delta)$ by the solvent, by the energy pooling process, and by quenching by O_2^- (which at the same time is depleted by back electron transfer). Fit of the integrated kinetic equation with a single adjustable parameter gave excellent agreement with decays observed in our concentration and laser pulse energy studies. The parameter chosen, r, is proportional to $[O_2^-]_o$, the superoxide concentration immediately after the photolysis laser pulse. A plot of r against the photolysis laser pulse energy E showed a close to quadratic dependence (slope of log r versus log E plot: 1.9 (CD_3CN) and 1.7 (CH_3N)). This indicates that most O_2^- is produced along a two photon excitation path: the first 266 nm photon excites the endoperoixde to the S_2 excited state, from which break up into $O_2(^1\Delta)$ and ground state dimethylnaphthalene occurs on a spin conserving singlet path at a yield of 25%. Since the adiabatic decomposition on the S_2 surface is fast compared to the 5 nanosecond duration of the 266 nm laser photolysis pulse, the naphthalene fragment has a chance to absorb a second photon. S_1 excited naphthalene so produced is capable of reducing $O_2(^1\Delta)$ with a driving force of over 2.5 volt (in acetonitrile), a process which we believe to be responsible for the appearance of superoxide. We have obtained direct spectroscopic evidence for the ionic endoperoxide photolysis products by detection of a transient dimethylnaphthalene cation radical absorption in the range 600-700 nm.

It is interesting to note that we did not find any evidence for expulsion of O_2 in the $^1\Sigma^+$ excited state, another possible spin allowed decomposition mode of S_2 endoperoxide. We have recently found a detection method for $O_2(^1\Sigma^+)$ in solution, namely monitoring the $^1\Sigma^+ \rightarrow ^1\Delta$ excited state fluorescence at 1.93 micron. A search for this near infrared chemiluminescence upon 266 nm photolysis of $DMNO_2$ did not yield any sign that singlet sigma O_2 is produced upon endoperoxide photodecomposition.

This work has revealed the processes that influence the decay of $O_2(^1\Delta)$ photoeliminated from a naphthalene type endoperoxide storage molecule. The results are of key importance for our study of the excited state chemistry of the retrieved $O_2(^1\Delta)$ by time resolved spectroscopy.

Singlet Oxygen Induced Redox Chemistry

In our search for aqueous $O_2(^1\Delta)$ excited state redox chemistry that permits direct conversion

of $^1\Delta$ electronic excitation into electrochemical potential, we have recently begun to explore oxidation of halides. A first example is the time resolved observation of the oxidation product I_3^- by transient absorption spectroscopy in the blue spectral range upon pulsed photosensitized oxidation of I^-.

$$O_2(^1\Delta) + 3I^- + 2H^+ \rightarrow I_3^- + H_2O_2(H_2O)$$

Observation of a factor of three increase in I_3^- yield upon replacement of H_2O by D_2O (at an I^- concentration of 0.15 M) gave direct proof for $O_2(^1\Delta)$ induced chemistry. This factor is equal to the lifetime difference of $O_2(^1\Delta)$ in H_2O and D_2O in the presence of 0.15 M I^-, as determined from the time resolved decay of the $O_2(^1\Delta)$ emission at 1.3 micron. Attempts to observe one electron transfer steps of this important reaction by near infrared chemiluminescence and transient absorption spectroscopy are under way.

Near Infrared Chemistry of SO

We are concentrating our search for low energy paths of new bimolecular reactions that can be accessed by near infrared photons on the chemistry of singlet sulfur monoxide. $SO(^1\Delta)$ has its potential energy minimum deep in the near infrared, at 5800 cm^{-1}, while the onset of the $^1\Sigma^+ \leftarrow ^3\Sigma^-$ progression lies at 10500 cm^{-1}. Reactions of these states are most conveniently studied by embedding SO-reactant pairs in an inert gas solid at cryogenic temperature. Under these conditions self reaction of ground state SO is inhibited, and wavelength selected photochemistry can conveniently be followed by monitoring the infrared product growth by FT-IR spectroscopy.

We have succeeded in trapping SO in Ar matrices by pyrolysis of thiirane oxide during matrix deposition. At about 400°C, over 50% of the episulfoxide decomposes to SO and ethylene without side reaction. Then, in order to study the reaction of singlet SO with ethylene, we have codeposited C_2D_4 through a separate deposition line. While SO isolated in Ar absorbs at 1139 cm^{-1}, sulfur monoxide with an ethylene nearest neighbor absorbs 12 cm^{-1} lower, at 1127 cm^{-1}. When irradiating these pairs with light in the range 5700-6000 cm^{-1}, the spectral region in which SO ($^1\Delta, v = O$) absorbs, we observed a decrease of the 1127 cm^{-1} $SO \cdot C_2D_4$ nearest neighbor absorption, while 10 new bands grew in. The product bands are tentatively assigned to perdeutero thiirane, C_2D_4SO. This constitutes the first observed chemical reaction of singlet excited SO in any phase. Attempts to locate higher vibronic levels of $^1\Delta$ and $^1\Sigma^+$ SO by reaction excitation spectroscopy, and exploration of other singlet SO reactions are in progress.

---

3. ARTIFICIAL PHOTOSYNTHESIS – Professor Melvin Calvin and Dr. John W. Otvos

The longterm ultimate solution of our needs for liquid fuels from renewable sources will be the construction of synthetic systems which will enable us to convert solar energy directly into useful chemical species, both as chemicals and for fuels. We are using what we know about the natural process of photosynthesis, insofar as it involves quantum conversion into stable chemical

products, to guide us in the design of these totally synthetic systems. The principal factor which enables green plants to convert incoming visible quanta into some stable chemical form long enough to do secondary chemical reactions for storage involves the separation of charge across a phase boundary. The positive charge (hole) is ultimately used to generate oxygen from water and the negative charge is used to reduce carbon dioxide. The phase boundaries under study are: (1) the lipid bilayer walls of phospholipid vesicles which are used to keep separate the initial photoproducts; (2) the surfaces of various polyelectrolytes; and (3) the surfaces of various colloids.

Efforts have been made to find microheterogeneous systems that would involve photosensitizers active in the visible region. These would promote electron transfer from donors to acceptors, which would stabilize the charge separation long enough for suitable catalysts to make use of their separation. The particular systems which have been most useful have involved sensitizers with relatively long-lived triplet states. The two components of such systems can be examined separately by using irreversible electron acceptors on the oxygen-generating side to stabilize the hole and irreversible electron donors on the hydrogen side to inhibit the back reaction of the initial charge separation.

Oxidation Catalysts

We are examining manganese porphyrin species as potential multi-electron oxidation catalyst for oxygen evolution from water and for other oxidation reaction. In photosynthesis some type of manganese complex is involved in the oxygen evolution process. Manganese porphyrin complexes exhibit a rich variety of oxidation states in which the porphyrin macrocycle is resistant to irreversible redox reactions. These properties make them promising oxidation catalysts, and, in addition, it has recently been shown that manganese porphyrin complexes catalyze the oxidation of olefinic hydrocarbons. Our research is directed at characterizing various highly oxidized manganese porphyrin species and studying their chemistry with the view of judging their potential usefulness in the oxidation cycle of an artificial photosynthesis assembly.

The work has proceeded along two parallel pathways. The first is directed at water soluble manganese porphyrins and involves chemical, electrochemical and photochemical studies. However, isolation of intermediate species is frequently easier in organic solvents, and we are also investigating the redox chemistry of manganese tetraphenylporphyrin complexes in organic media. Comparison of similarities and differences in the properties of oxidized manganese porphyrins in aqueous and nonaqueous systems has led to helpful insights and has suggested new experiments.

An effort is underway to prepare a polynuclear manganese complex with some cyclic amines, i.e. cyclams. There is reason to suspect that the multiple manganese sites on such a molecule could provide the capability of bringing two oxygen atoms together to form O_2.

Electrocatalytic and Photochemical Reduction of CO_2

We have continued to study the electrocatalytic reduction of carbon dioxide with cobalt and nickel macrocycles as catalysts in aqueous solution. We have found that of the various tetra-aza compounds tried as catalysts the ones that have given the best results in electrode reduction of CO_2 have unsubstituted, saturated ring structures. One of these [1,4,8,11-tetra–aza cyclotetradecane nickel (II)] was found to be highly selective in producing CO rather than H_2, as well as giving almost 100% current efficiency and high turnover numbers on Ni(II). After we were able to repeat these results, we selected this catalyst as the basis for a three-component photochemical system for performing the CO_2 reduction. Ruthenium tris-bipyridyl [$Ru(bipy)_3^{2+}$] was the sensitizer and ascorbate buffer the electron donor. Work is now in progress to find the conditions that will maximize the yield of CO and also to analyze the aqueous phase of other possible reduction products of CO_2.

Polyelectrolytes and Charged Colloids as Interfaces for Increasing Quantum Efficiency

We have continued our studies on the effect of charged interfaces on the photoelectron transfer reaction between the photosensitizer and the electron acceptor. After our initial results with the polyelectrolyte, polystyrene sulfonate (PSS), showed that its presence not only inhibited the back reaction, as does colloidal silica, but also repressed the forward reaction and thus had little effect on the overall quantum yield, we began work with modified silica colloids. However, the usefulness of silica is limited to pH regions above 8. If sodium aluminate is used to react with the surface of the sol, aluminum can be incorporated into the surface. The more acid aluminum sites make the colloid stable and usable down to pH 6 where it still retains all of its effectiveness in preventing back-reaction.

Membranes

In order to achieve photoelectron and hole separation we have proposed to use electron transferring membranes which will keep the oxidizing and reducing agents separated. We have been working with perfluorinated ion exchange membrane (Nafion) obtained from duPont as well as membranes from the Dow Chemical Company. The objective here is to load such a perfluorinated cation exchange membrane with a cationic redox compound and so construct an electron transfer membrane by virtue of electron hopping between the two states of the redox couple bound to the membrane.

PUBLICATIONS – CHEMICAL SCIENCES

1. P.-T. Chou and H. Frei. Time-resolved Decay of $O_2(^1\Delta_g)$ Expelled from 1,4 Dimethylnaphthalene Endoperoxide: A Laser Flash Chemiluminescence Study. J. Chem. Phys., in press.
2. P.-T. Chou, S. Khan, and H. Frei. Time Resolved $O_2{}^1\Delta_g \rightarrow {}^3\Sigma_g^-$ Chemiluminescence upon UV Laser Photolysis of an Aromatic Endoperoxide in Aqueous Solution. Chem. Phys. Lett., **129**, 463 (1986).
3. K. Consani and G.C. Pimentel. Infrared Spectra of the Clathrate Hydrates of Acetylene and of Acetylene/Acetone. J. Phys. Chem. **91**, 389 (1987).
4. H. Frei. Chemistry with Near Infrared Photons. Proceedings of the U.S.-Japan Cooperative Photoconversion/Photosynthesis Seminar, Honolulu, March 1987.
5. H. Frei and G. C. Pimentel. Chemistry on Ground and Excited Electronic Surfaces Induced by Selective Photo-excitation in Matrices. **In**: Chemistry and Physics of Matrix Isolated Species, (L. Andrews and M. Moskovits, Eds.), North Holland Publishing Company, Amsterdam, in press.
6. E. Orton, S.T. Collins and G.C. Pimentel. Molecular Structure of the Nitrile Ylide Derived from 3-Phenyl-2H-azirine in a Nitrogen Matrix. J. Phys. Chem. **90**, 6139 (1986).
7. R. Rafaeloff, A.C. Maliyackel, J.L. Grant, J. W. Otvos and Melvin Calvin. Photoinduced Behavior of Zinc and Manganese Porphyrins in Dihexadecyl Phosphate and L-α-Phosphatidyl-DL-Glycero. Vesicular Systems. Nouv. J. Chim. **10**, 613 (1986).
8. Carl C. Wamser, Melvin Calvin and Gregor A. Graf. Preparation and Properties of Porphyrin-Modified Hollow-Fiber Membranes for Use as Heterogeneous Photosensitizers for Singlet Oxygen and for Artificial Photosynthesis. J. Membrane Sci. **28**, 31 (1986).

KCO6 – BIOLOGICAL ENERGY RESEARCH

The research in this program is aimed at understanding the unique features of photosynthetic organisms that allow them to collect light energy and store it in the form of chemical energy.

- One project utilizes spectroscopic techniques to map both the components as well as the kinetics of the light reactions. The focus is on excitation transfer and trapping, primary electron transfer, the composition and organization of photosynthetically active membranes.

- We are studying the genetics of the photosynthetic apparati of *Rhodopseudomonas capsulata* and *Euglena gracilis* with the ultimate aim of using DNA cloning techniques to help understand the mechanisms cells use to capture and utilize light energy.

- Our goal in a third project is to identify the cellular factors which regulate the tissue specific expression of plant genes.

- A complete understanding of the chemistry and stereochemistry of phycobiliproteins and phytochrome is sought to facilitate full understanding of the role of light in regulation of gene expression in green plants.

- A fifth program seeks to develop an understanding of the process involved in hydrocarbon production in plants.

1. PHOTOSYNTHESIS REACTIONS – Professor Kenneth Sauer and Dr. Melvin P. Klein

Photosynthetic light reactions constitute the principal biological energy source derived from sunlight. We are carrying out spectroscopic and other biophysical studies aimed at understanding the mechanism of photon capture and excitation transfer among photosynthetic pigments, the earliest steps in conversion to chemical potential by electron transfer in the reaction centers and the mechanism of water oxidation to O_2. Both kinetic and structural investigations are carried out using a variety of spectroscopic techniques, including optical absorption and fluorescence, electron paramagnetic resonance (EPR) and X-ray spectroscopy. In related studies we are looking at the composition and organization of the photosynthetic complexes and their arrangement in the thylakoid membranes. We have also investigated model compounds designed to simulate the light-induced electron transfer that lies at the heart of the photosynthetic energy conversion.

Excitation Transfer in Photosynthetic Antenna Pigments

We have used time-resolved fluorescence relaxation measurements to monitor the excited state lifetimes and excitation transfer dynamics in photosynthetic antenna pigments. A particularly interesting set of these pigments occurs in the phycobiliprotein complexes that serve to absorb photons of visible light and to transfer the resulting excitation to photosynthetic reaction centers. We calculated excited state lifetimes for the pigment C-phycocyanin using the inductive resonance transfer method of Förster [Sauer, Scheer and Sauer, Photochem. Photobiol., in press] and based on X-ray crystallographic coordinates published by Schirmer, et al [J. Mol. Biol. **188**, 651-657 (1986)]. Satisfactory agreement between the calculations and experimental measurements indicates that this mechanism largely accounts for the excitation transfer in C-phycocyanin. We have now extended these studies to the allophycocyanin-containing core complexes from phycobilisomes. In this case spectroscopic evidence indicates that exciton coupling plays a major role in excitation transfer. To test whether the much faster kinetics associated with the exciton mechanism can be detected, we are planning to construct a sub-picosecond pulsed dye laser spectrometer.

The chlorophyll pigments associated with photosynthetic reaction centers are clustered in several spectroscopically distinct protein complexes. Using steady-state as well as time-resolved fluorescence measurements, we have looked at the temperature dependence of the fluorescence associated with Photosytem I reaction center complexes. A dramatic increase in fluorescence that occurs at long wavelength at low-temperatures produces a relatively long lifetime (2.5 nsec) decay component. This presumably reflects excitation that is unable to reach the reaction center, which lies somewhat higher in energy than the excited state of the fluorescent molecule(s). In fact, an Arrhenius plot of the fluorescence intensity against temperature gives an activation energy that corresponds to the difference between the energies of the antenna pigment and that of the reaction center. The occurrence of low-lying excited states in pigment molecules closely associated with reaction centers appears to be widespread among photosynthetic organisms. The role of these pigments is not clear at present; however, they are involved in providing a thermal assist to those absorbed photons at the red end of the spectrum that do not have quite enough energy to activate the reaction center directly. This could have a significant effect in increasing

the efficiency of photosynthetic solar energy conversion.

Structure and Function of Photosynthetic Reaction Centers

Much new information about the organization of photosynthetic reaction center complexes has accumulated following the determination by X-ray crystallography of the structure of the complex from *Rhodopseudomonas viridis* [Diesenhofer, et al., Nature **318**, 618-624 (1985)]. Strong homologies in the amino acid sequences of two peptides from the reaction centers of Photosystem II of higher plants or cyanobacteria with those of several purple bacteria, together with similarities in electron transport cofactors, are indicative of close similarities in the structures of these complexes from widely different photosynthetic organisms. One common feature is the occurrence of quinones as electron acceptors in these reaction centers. Using a chemically reactive quinone we have succeeded in specifically labelling a polypeptide (D2) of the Photosystem II reaction center complex that bears sequence homology to the M subunit polypeptide of reaction centers from purple bacteria [Worland, Yamagishi, Isaccs, Sauer and Hearst, Proc. Natl. Acad. Sci. USA **84**, 1774-1778 (1987)]. Previous evidence implicated the companion peptide, D1, in reactions at the electron acceptor side of the Photosystem II reaction center. In addition, there are experiments in the literature that point to a role on the electrón donor side of the PSII reaction center for the D1 and D2 polypeptides. We have used this clue to begin an investigation of potential binding sites for the manganese complex that is involved in photosynthetic oxygen evolution. This study ties in with the measurements of spectroscopic properties of the oxygen evolving complex that we have carried out during the past decade.

Photosystem I in higher plants and cyanobacteria contains reaction centers that are dissimilar to those of PSII or of purple bacteria. They contain as electron acceptors a set of three iron-sulfur proteins that operate at unusually low electrochemical potential. Although EPR spectra of these iron-sulfur centers have been known for many years, little is known of their structure or of what distinguishes them as low potential electron acceptors. We have carried out X-ray spectroscopic investigations of the iron in these complexes using the facilities of the Stanford Synchrotron Radiation Laboratory. K-edge absorption spectra are very similar to those of the related ferredoxin molecules, and EXAFS spectra indicate that the three iron-sulfur centers contain a mix of 2Fe2S and 4Fe4S complexes [McDermott, et al., in **Progress in Photosynthesis Research** (ed. Biggins), vol. I, pp 249-252, Martinus Nijhoff, Dordrecht, 1987]. Through collaboration with John Golbeck at Portland State University we are seeking to extend these studies by investigating preparations where the iron-sulfur proteins are separated from one another.

Manganese and Photosynthetic Water Oxidation

Much evidence has now accumulated that the storage of the four oxidizing equivalents needed to produce O_2 from water photosynthetically occurs in a complex containing manganese atoms. The nature of this complex, which is presumed to involve interaction with a protein environment, is still unknown. Even the kinetics is in dispute. Optical spectroscopic changes have been interpreted to result from three successive one-electron oxidations from Mn^{+3} to Mn^{+4} prior

to O_2 release, whereas EPR, X-ray spectroscopy and other approaches favor a mechanism with different oxidation state changes for the three preliminary steps.

We have used X-ray near edge spectroscopy and EXAFS in conjunction with low temperature EPR measurements to investigate these questions. Absorption edge energy studies on Mn are convincing that an S_0-like state, the most reduced state of the complex, has a lower oxidation state than has S_1. The second step, from S_1 to S_2, definitely involves another Mn oxidation. Samples illuminated at 140K and observed at 4.2K exhibit an ESR signal with g = 4.1. The Mn X-ray edge position for this state is identical to that from samples prepared by illumination at 190K and by illumination at 277K in the presence of DCMU, a reagent that limits the PSII reaction center to a single turnover. The third step, from S_2 to S_3, does not appear to involve further oxidation of Mn. Presumably this electron has come from an associated ligand that does not directly involve Mn. The final step,from S_3 to S_4 and back to S_0 returns the complex back to the fully reduced state. The state S_4, which should be the most oxidized but is unstable, has not yet been trapped for investigation.

A pre-edge feature, usually assigned to transitions between the 1s and 3d levels in the Mn X-ray absorption spectrum, has been studied in numerous model systems as well as in the Photosystem II preparations. All model compounds in which the Mn atoms are in the +III oxidation state exhibit a broad pre-edge feature with splittings of some 2.5 eV. By contrast, those complexes containing Mn(IV) exhibit a narrow more intense pre-edge feature with splittings of 1.8 - 2.3 eV. Photosystem II particles from both spinach and the cyanobacterium Synechococcus in the S_1 state correspond to the Mn(III) models while those in the S_2 state correspond closely to the Mn(IV) models. Theoretical interpretations of these differences are current topics of investigation. The most likely source is the Jahn-Teller effect in the high spin Mn(III) complexes.

EXAFS measurements give information about the local coordination environment of the Mn in the water splitting complex. Two of the three shells previously identified for S_1 are attributed to O or N atoms, and the third shell to Mn atoms with an admixture of other low Z atoms, presumably O or N. The data lead to a structure in which a pair of Mn atoms, separated by 2.7Å, is coupled via μ-oxo ligands at 1.8Å. The structures in the S_1, S_2 and S_3 appear to be virtually identical. The structure in the S_0^* state appears to be somewhat different with a slight increase in the Mn-Mn distance.

The content of Mn in the Photosystem II preparations is generally agreed to be four atoms per PSII reaction center. Our EXAFS results are consistent with pairs of Mn atoms although in some data sets we have indications of an additional shell of scatterers in the radial distribution. The structures determined by EXAFS are not consistent with a regular adamantane with Mn-Mn distances of some 3.3Ånor are they compatible with a regular cubane-like structure. Both have been proposed in the literature.

There are three extrinsic proteins with molecular weights of 16, 23 and 33 kDa associated with the oxygen-evolving complex. It has been proposed that the 33 kDa species may provide ligands to the Mn cluster. Treatment of PSII particles with high concentrations of $CaCl_2$ release all three peptides with retention of Mn. Subsequent incubation in low ionic strength media releases two

of the four Mn. X-ray absorption edge and EXAFS measurements of the high salt preparations demonstrate that the oxidation state and ligand environment of the Mn is unchanged from the untreated particles. PSII particles from which two Mn atoms have been removed exhibit a marked lowering in the absorption edge energy, suggesting reduction, and a drastically altered ligand environment including loss of the μ-oxo bridged binuclear structure.

To further probe the structure of this Mn complex we have constructed a spectrometer to perform pulsed or time domain Electron Spin Resonance spectroscopy. The electron spin echo spectra exhibit modulation resulting from interactions between magnetic nuclei and the electron spin. Fourier transformation and subsequent simulation of these modulations can identify the nuclear moieties and their interactions. The complex ESR signal corresponding to the S_2 state has been examined with this new instrument with several important results. The multiline signal has been attributed to at least a pair of interacting Mn atoms. We find that some hydrogen atoms within the magnetic coordination sphere of this moiety can be exchanged for deuterium. There is a contribution from a few nitrogen atoms. Upon incubation with NH_3, a known water analog, deep nitrogen modulation occurs demonstrating direct nitrogen ligation to Mn. We have demonstrated that this multiline ESR signal can be observed at 1.5K and shows Curie-like behavior between 4.2K and 1.5K. Such observations clearly contradict previous reports that the multiline signal arises from an excited S=1/2 state.

---

2. MOLECULAR GENETICS OF PHOTOSYNTHESIS GENES IN *Rhodobacter capsulatus* – Professor John E. Hearst

The R-prime plasmid, pRPS404, contains a 46kb segment of *R. capsulatus* DNA with coding information for photosynthetic proteins. It contains the tightly linked genes *puf*B, *puf*A, *puf*L and *puf*M coding for the light-harvesting I β and α proteins and the L and M reaction center proteins, respectively. It also contains the unlinked gene *puh*A which codes for the reaction center H protein. Between the *puf* and *puh* loci is a long stretch of DNA with many genes for carotenoid and bacteriochlorophyll biosynthesis enzymes. The long term goal of this task is a complete understanding of this photosynthetic gene cluster, including a complete map of the genes contained on this piece of DNA, a complete nucleotide sequence of the entire gene cluster, an understanding of the enzymatic activity associated with each gene, and an understanding of the regulation of each of these genes especially with respect to light intensity and oxygen concentration.

Youvan et al (1984a,b) reported the sequence of two restriction fragments from the photosynthetic insert of the R-prime plasmid, pRPS404, which contain the *puf* and *puh* loci. *Puf* is polycistronic and transcribed from two oxygen-repressed promoters upstream from the genes in *puf*. The *puh* loci is 35kb away and may be polycistronic as well. It also is transcribed from at least one oxygen-repressed promoter. It is clear that their is coordinate regulation of these two operons, but the mechanism for this coordination is not understood.

Comparison of the *R. capsulatus* M subunit sequence and the *Rps.sphaeroides* M subunit sequence shows that the entire polypeptide sequence has been highly conserved (76.5% hology).

There is also impressive homology between the L and M subunits of these bacteria and the QB protein of spinach. We now believe that all PSII reaction centers contain two similar protein subunits with molecular weights between 30 kDa and 40 kDa with roles analogous to those of the L and M subunits. The genes for these PSII proteins in higher plants are at the *psb*D and the *psb*A loci in chloroplasts genomes. The corresponding proteins have been designated D1 and D2.

The genetics of photosynthetic genes has been greatly extended by the use of transposon mutagenesis of the gene cluster in pRPS404. The transposon mutagenesis work with Tn5.7 identified a large number of genes and increased the resolution of the gene map. This transposon map is the beginning of most of the new mapping and sequencing studies reported in this task.

Photosynthesis in *Rhodobacter capsulatus* occurs in the absence of oxygen and the presence of light. Under these conditions, cells develop an extensive intracytoplasmic membrane system containing the photosynthetic apparatus. The isolation and sequencing of the genes coding for the light harvesting I (LHI) and reaction center (RC) polypeptides (Youvan, et.al., 1984b) makes the development of a purified transcription system attractive as a first step in studying the molecular mechanisms regulating the production of these photosynthetic complexes.

Recent results in both *R. capsulatus* (Zhu and Hearst, 1986) and a closely related species, *R. sphaeroides* (Zhu and Kaplan, 1985), indicate that steady state levels of messenger RNA coding for LHI and RC complexes increase 4- to 10-fold when the oxygen tension of growth medium drops below about 2 percent. Polypeptides corresponding to these transcripts are not detectable by immunoblotting techniques in aerobically grown cells, but show a rapid buildup in cellular membranes within 1 hour of the shift in oxygen tension (Klug, et.al. 1985; Chory, et.al., 1984). These results imply transcriptional and translational control of photosynthetic gene expression.

At the transcriptional level, oxygen partial pressure is the primary environmental factor controlling expression. Reduction in oxygen tension results in an approximately 4-fold increase in messenger RNA; a subsequent decrease in light intensity stimulates transcription another 2-fold (Zhu and Hearst, 1986). Unpublished reports indicate that 5-prime regions of DNA responsible for these effects have been identified for the LHI operon (coding for LHI and RC polypeptides). The purification of an RNA polymerase capable of transcribing these genes *in vitro* will lead to a more detailed understanding of how the photosynthetic apparatus is regulated. Other proteins are probably also involved in this process. Having a purified polymerase will also be useful as a tool to isolate these accessory transcriptional factors.

Regulation of Gene Expression

Regulation of photosynthetic gene expression in *Rps. capsulata* has been studied using a series of transposon insertions mutations within the photosynthetic gene cluster. Insertions within the structural genes for the reaction center polypeptides have been shown to be pleitropic. Such mutants show reduced absorption of the LHII complex, reduced mRNA levels for the reaction center and LHI polypeptides, reduced levels of reaction center proteins, and inhibition of bacteriochlorophyll biosynthesis. The same phenotype is observed in mutants defective in

bacteriochlorophyll biosynthesis. These results suggest that there is a tight coupling between expression of the genes for biosynthesis of bacteriochlorophyll and the reaction center and LHI proteins. A tentative model has been presented in which the reaction center H subunit, by virtue of its role in mediating formation of an intermediate complex within the membrane, represents a central element in control of photosynthetic differentiation. At a recent meeting in Freiburg, we introduced the model of a membrane bound synthesome associated with the formation of reaction centers.

For the LHI genes we have found that there are two transcripts (0.5 and 2.6 kb), while the LHII genes only have one transcript (0.5 kb). The 0.5 kb transcripts of both genes are most abundant. The level of LHII 0.5 kb transcript is more sensitive to change in oxygen concentration and shows a variation over a wider range than that of the LHI, indicating that the LHII and LHI/RC genes are regulated independently. The *puh* loci has been found to have at least two transcripts (1.4 and 1.2 kb) which originate in the open reading frame (ORF 1696) and which respond differentially to light intensity. We have shown that increased light intensity causes decreases in the expression of the genes for LHI, LHII, RC, and Bchl biosynthesis genes; however, it results in increased expression of the genes for Crt biosynthesis. These results are interpreted in terms of the protective function of the carotenoids to photo-oxidation.

Oxygen regulation of photosynthetic genes is mediated by supercoiling of DNA in *Rhodobacter capsulatus*

The regulation of the photosynthetic genes by DNA supercoiling in *Rhodobacter capsulatus* has been studied by using gyrase inhibitors and *in vivo* measurement of mRNA. The results demonstrate that the biosynthesis of bacteriochlorophyll is markedly repressed by gyrase inhibitors, novobiocin, coumermycin, nalidixic acid and oxolinic acid under both anaerobic conditions or during a shift from aerobic to anaerobic conditions. Coumermycin exhibits the strongest inhibitory effect and the inhibition is non-reversible in contrast to the other inhibitors whose inhibitory effects can be reversed by washing them out from the cells. The synthesis of light harvesting (I, II) bacteriochlorophyll complexes were also inhibited by novobiocin and coumermycin. It has been further shown that the levels of mRNA for light harvesting (I, II), reaction center (L, M, H), bacteriochloropyll biosynthetic enzymes, ribulose-bis-phosphate carboxylase and a putative regulatory factor (Q) decreased rapidly and greatly upon addition of novobiocin and coumermycin. Contrary, the mRNA for carotenoid biosynthetic enzymes and rRNA were less sensitive to the inhibitors. The results, coupled with studies on bacteriochlorophyll and light harvesting complexes, strongly suggest that DNA supercoiling is involved in the differential expression of photosynthetic genes in response to oxygen in *R. capsulatus*.

Characterization of Carotenoid Synthesis Genes

Single strand DNA of *Bam*J M13 subclones have been isolated and characterized using the C test for hybridization, which gives information about the relative directionality of the insert DNA. This single stranded DNA has been used in sequencing studies.

With improvements in RNA isolation procedures, RNA-DNA hybridization techniques, and availability of more specific probes, it has been possible to characterize the small, abundant *Bam*J transcript. Refined measurements of RNA size show this transcript to be about 400 bp long. Because of its size, this RNA initially was a candidate for the transcriptional product of the small *crt*F gene which has been mapped to the *Bam*J. A Southern blot from a triple restriction digest of *Bam*J localized the region of heavy transcription to 611 bp HindIII-SphI restriction fragment, which appears to be separated from the putative *crt*F gene by roughly 1.3 kb. Extensive probing with M13 subclones has localized the boundaries of this mRNA.

In efforts to clarify the identity of the 400 bp transcript, additional experiments have shown that this RNA is absent in an unpigmented mutant arising from the spontaneous deletion of a large piece of DNA, following transposon insertion. This mutant retains the ability to grow aerobically via respiration; the transcript, therefore, does not code for a unique, essential respiratory function. Preliminary dot blots and Northern blots with RNA from a *crt*F point mutant indicate no reduction in the levels of the small transcript. Additional experiments using *crt*F mutants will be done to verify this result. Some earlier mutations in the carotenoid biosynthetic pathway may reduce transcription levels. Dot blots probed with DNA within the *Bam*J fragment and adjacent to the *Bam*M fragment indicate complete loss of transcription in this region in a *crt*E transposon mutant.

A second line of experiments has shown that carotenoid mutants blocked in various stages of pigment biosynthesis exhibit differential reduction (up to 5-fold) of the mRNAs for the *puf*, *puh*, and *puc* operons. In the case of the *puc* operon, reductions in mRNA appear to correlate well with reductions in absorption due to LHII in chromatophore preparations. Thus, carotenoid genes interact with genes for the structural photosynthetic proteins at the level of gene expression.

Finally, the level of steady-state mRNA from the carotenoid genes has been examined by Southern blotting of restricted DNA containing these genes, probed with RNA from photosynthetic cells. We conclude that the crt mRNA levels are at most 1% that of mRNA for the *pufA,B* genes of LHI.

References

1. Chory, Donohue, Varga, Staehelin, and Kaplan (1984) J. Bact. 159, 540-554
2. Klug, Kaufmann, and Drews (1985) Proc. Nat. Acad. Sci. USA 82, 6485-6489
3. Youvan, Alberti, Begusch, Bylina and Hearst (1984a) Proc. Nat. Acad. Sci. USA 81, 189-192
4. Youvan, Bylina, Alberti, Begusch and Hearst (1984b) Cell 37, 949-957
5. Zhu and Hearst (1986) Proc. Nat. Acad. Sci. USA 83, 7613-7617
6. Zhu and Kaplan (1985) J. Bact. 162, 925-932
7. Zhu, Kiley, Donohue, and Kaplan (1986) J. Biol. Chem. 261, 10366-10374

3. LIGHT REGULATION OF NUCLEAR AND CHLOROPLAST NUCLEIC ACID SYNTHESIS IN *Euglena gracilis* – Dr. James C. Bartholomew

Our research has been directed towards the understanding of the light control of expression of the many genes encoding the photosynthetic apparatus in the eucarotic alga *Euglena gracilis*. While the photosynthetic processes are primarily carried out in the chloroplasts of eucaryotic photosynthetic cells, the genes encoding the components are distributed into both the nuclear and the chloroplast genomes. The mechanism(s) coordinating the expression of these physically separated genomes is not known. In most eucaryotic cells the nuclear pattern of gene expression is linked to the position of the cells in the cell cycle. In *Euglena* light has a dramatic effect on cell cycle traverse. *Euglena* placed on a 12 hr light – 12 hr dark regime will become synchronized in their traverse of the cell cycle. The relationship between the cell cycle response to light and the photosynthetic gene expression as a function of light is the subject of our investigations. Is the nuclear and chloroplast gene expression program linked to traverse of the cell cycle in this photosynthetic organism, or are the photosynthetic genes regulated independently of cell cycle traverse. We are characterizing the cell cycle response to light of *Euglena gracilis* by flow cytometry. Wild type Euglena grown to saturation density can be stimulated by light to reenter the cell cycle. We are comparing the kinetics of reentry into the cell cycle after different periods of light exposure. Our studies have shown that *Euglena* requires at least 6 hrs of light exposure to reentry in the cell cycle. To monitor the expression of genes during the cell cycle we have used probes for the photosystem II reaction center herbicide–binding protein, *psbA*, and the large and small subunits of ribulose 1,5–bisphosphate carboxylase, *rbcL* and *rbcS*. We are also developing a probe for the light harvesting binding protein *LHCP*. All of the messanger RNA'es for these genes accumulate in a light dependent fashion with a maximum after 6 hr light exposure. Even cells at saturation density show a peak expression of these messages after 6 hrs exposure to light, however, the maximum expression of the messages is much less than cells which are traversing the cell cycle. These results indicate that these genes for photosynthetic components are regulated by light independent from the cell cycle, but that the progress of cells around the cycle can affect the message levels quantitatively.

---

4. TISSUE-SPECIFIC EXPRESSION OF Ri T-DNA GENES IN *Nicotiana tabacum* – Dr. Francesca Leach and Dr. James C. Bartholomew

Project Description

The project has been designed to identify cytoplasmic factors which are responsible for specific gene expression in two different plant tissues: leaves and roots. Only a few instances of proteins controlling the activity of plant promoters by binding to upstream DNA sequences, just like in bacteria and in mammalian cells, have been identified. This has been the case in the control of zein gene expression. The study of how transcription is controlled in plants is crucial in an era in which plant genetic engineering is becoming possible. It is essential that any foreign gene which is introduced in plant tissues can be expressed in a regulated manner if desired.

Accomplishments

We are working on several novel techniques to assay and visualize DNA-protein complex formation between leaf or root cellular extracts and portions of the pRi T-DNA. These are filter-binding assays and gel retardation techniques.

We have made several DNA constructs, where the acetyl-chloramphenicol transferase gene from Tn 9 has been fused to the Ri root or leaf specific promoters. The constructs have all been sequenced to test for the orientation of the CAT gene in regard to the Ri promoters. The aim is to test for the transient CAT activity after transformation of *Nicotiana tabacum* leaf or root protoplasts. We are expecting the leaf protoplasts to contain the necessary factors to activate the leaf promoter, but not the root promoter. We are hence expecting to observe CAT activity after transformation of leaf protoplasts by the pRi T-DNA leaf-promoter/CAT construct, but not by the root-promoter/CAT construct, and reciprocally after transformation of root protoplasts. We are testing the length of 5' upstream sequences necessary for activity of these two pRi promoters. We have established the optimal conditions for protoplast isolation, cell suspension cultures, electroporation and PEG transformation. We are now testing CAT acvtivity from the CAT plasmids constructed.

We have tested conditions that will allow plant protein-DNA complex formation. Tobacco root- and leaf-protein extracts have been tested for their DNA-binding capacities after separation of the proteins by electrophoresis and transfer to a nitrocellulose membrane. A number of proteins which show DNA-binding capacities have been observed, and large differences in the DNA-binding pattern of extracts from the two tissues were noted.

We are hoping to isolate, if they exist, proteins which bind specifically with the regulatory sequences of the root-specific and the leaf-specific T-DNA genes.

To visualize DNA-binding proteins we transfer proteins to nitrocellulose filters and probe with nick-translated labelled DNA. The DNA sequences we are using for these experiments are subclones containing the leaf-specific and the root-specific promoters of the *A. rhizogenes* T-DNA. The goal is to show that there is indeed a specificity in the recognition of each of these sequences by the two types of extracts and to identify what proteins are involved.

We are expecting difficulties in this part of the project in defining conditions that will enhance the sequence specificity of the DNA-binding proteins. This has been a major difficulty in the identification of sequence-specific recognition factors. Retarded electrophoretic migration of DNA complexed to proteins has proven to be a useful method in detecting specific over non-specific binding of proteins to isolated sequences. It has recently been used to identify specific transcription factors in SV40 infected cells, and in O_2 induced cytochrome expression in yeast.

We are now testing several approaches in order to determine the general and sequence-specific DNA-binding properties of the Tobacco root and leaf proteins. These include investigating factors influencing the formation of DNA-protein complexes in solution, such as salt conditions, length of reaction and subsequent washes, and competition experiments with random sequence DNA such as calf-thymus DNA.

Transformed cells obtained from the transient expression experiments will be cultured *in vitro* and plants containing these modified Ri T-DNAs will be regenerated. It is hoped that by adding extracts from leaf tissues to root nuclei and harvesting the subsequent transcription complexes it will be possible to activate the silent leaf-specific promoter (and vice-versa for the leaf-nuclei). The assay will be used to test the factors isolated as tissue-specific activators in the DNA binding work described above.

---

5. PLANT BIOCHEMISTRY – Professor H. Rapoport

The objectives of this project are to study the structure and mechanism of action of the non-chlorophyllous plant protein-pigment phytochrome. Phytochrome is the morphogenically active plant pigment that controls all developmental aspects of plant growth. It does this by undergoing a photochemical interconversion upon light excitation to a new, active form.

The specific structural features of this isomerization, as well as some other structural aspects of phytochrome, remains unknown. It is essential that these structural questions be unambiguously established in order to have a full understanding of phytochrome's function. Understanding the underlying mechanism by which phytochrome functions may allow us to control growth, flowering, and fruiting.

The specific aims of this project are:

1. To determine the stereochemistry of the pigment-protein linkage in phytochrome.

2. To determine the structural changes both in pigment and protein accompanying the change of phytochrome from the inactive P_R form to the active P_{FR} form.

3. To establish the relative and absolute stereochemistry of the bilipeptide linkages.

4. To synthesize the specific stereoisomers of S-cysteinylphytochromobilin.

5. To synthesize models of phytochrome consisting of pigment covalently attached to polypeptides of various lengths and composition in order to probe pigment-protein interactions.

Phytochrome is the morphogenically active plant chromoprotein intimately involved in all developmental aspects of plant growth. Because of its complex and sensitive nature, it has also eluded unambiguous structural assignment. However, the data obtained so far indicate it is similar to the phycobilins. We have applied new structural methodology to this extremely important plant pigment as well and have established the structure of the phytochromobiliundecapeptide.

Phytochrome is a reversible biological switch. The action of far red light causes it to change from the inactive P_R form to the active P_{FR} form. The two most reasonable hypotheses for its mode of action in the P_{FR} form are through gene activation or by affecting membrane permeability. However, structural information on exactly what changes accompany the transformation to the

P_{FR} form is totally lacking.

We plan to provide information about the P_R to P_{FR} transformation by applying our structural methodology to the P_{FR} form. We also plan to conduct the P_R P_{FR} reaction in a ^2H_2O medium and reisolate the P_R form. Detailed NMR analysis, both ^1H and ^{13}C, will then reveal if any hydrogen atoms have been exchanged in this process.

Our initial synthetic goal is S-cysteinylphytochromobilin. After this is successfully completed, the synthetic work will be extended to include some small chromopeptides. The synthetic work is essential because it will help us answer some questions about pigment conformation and function. Also, it will answer the final stereochemical question left unanswered in that it will establish the absolute stereochemistry at the thioether linkage.

Our proposed synthesis of S-cysteinylphytochromobilin begins with the protected L-vinylglycine ***1*** which we have recently prepared from methionine. With HBr in the cold this gives a mixture of about equal parts of the diasteriomeric bromides ***2*** and ***2'*** which are separable.

The synthesis now proceeds with each separate diastereomer in a displacement with a suitably N and O protected cysteine to ***3***. The α-amino group of ***3*** is now replaced by Br to give ***4*** and thence with cyanide to ***5***. Cyanoacetate ***5*** is alkylated with α-bromopropionate and the resulting ***6*** is decarboxylated to a mixture of ***7*** and ***7'*** in which the C*'s are of fixed and known stereochemistry. We now proceed individually with ***7*** and ***7'*** to ***8*** (and ***8'***. Thus in ***8*** all three centers are fixed and known, C-2 and C-3' by synthesis and C-3 by the splitting constant of its H with C-2-H.

From ***8*** we will continue the synthesis to the phytochrome bile pigment ***9*** and we can then turn to extension of the peptide chain from both the amino and carboxy ends. We shall not present the details of the peptide synthesis methodology here, since we expect to rely heavily on known methods.

Compound ***8*** has been prepared by two different paths in an optically inactive form as a mixture of various stereoisomers. We are now evaluating and improving these processes in order to select the method best suited for preparing optically active pure compound ***8***. We have prepared the compound corresponding to the two central rings (B and C) of compound ***9***.

Preliminary synthetic experiments starting with optically active vinylglycine ***1*** have yielded promising results, in that one isomer of ***8*** seems to be the major product. If this isomer has the stereochemistry of natural phytochrome, the synthetic prospects are very promising.

At the same time, we have started investigation of an alternative and shorter path via compound ***10***, synthesized in its sterically pure form from L-alanine. Initial experiments demonstrated that suitably protected cysteine derivatives undergo 1,4-addition to ***10*** to give ***8*** as a mixture of diastereomers. Furthermore, the same addition reaction occurs with the vinyl analogue of the A-B ring dimer. In the latter case, the diastereomers have been separated and are crystalline.

During the next year we will be synthesizing optically active pure compound ***8*** and will synthesize ring D of compound ***9***. Once these compounds are available we will proceed with the synthesis of compound ***9***. Emphasis will be placed on the newly discovered route involving 1,4-addition of a protected cysteine derivative.

Following the completion of synthesis of S-cysteinylphytochromobilin, the extension of the peptide chain from both carboxyl and amino ends will be carried out.

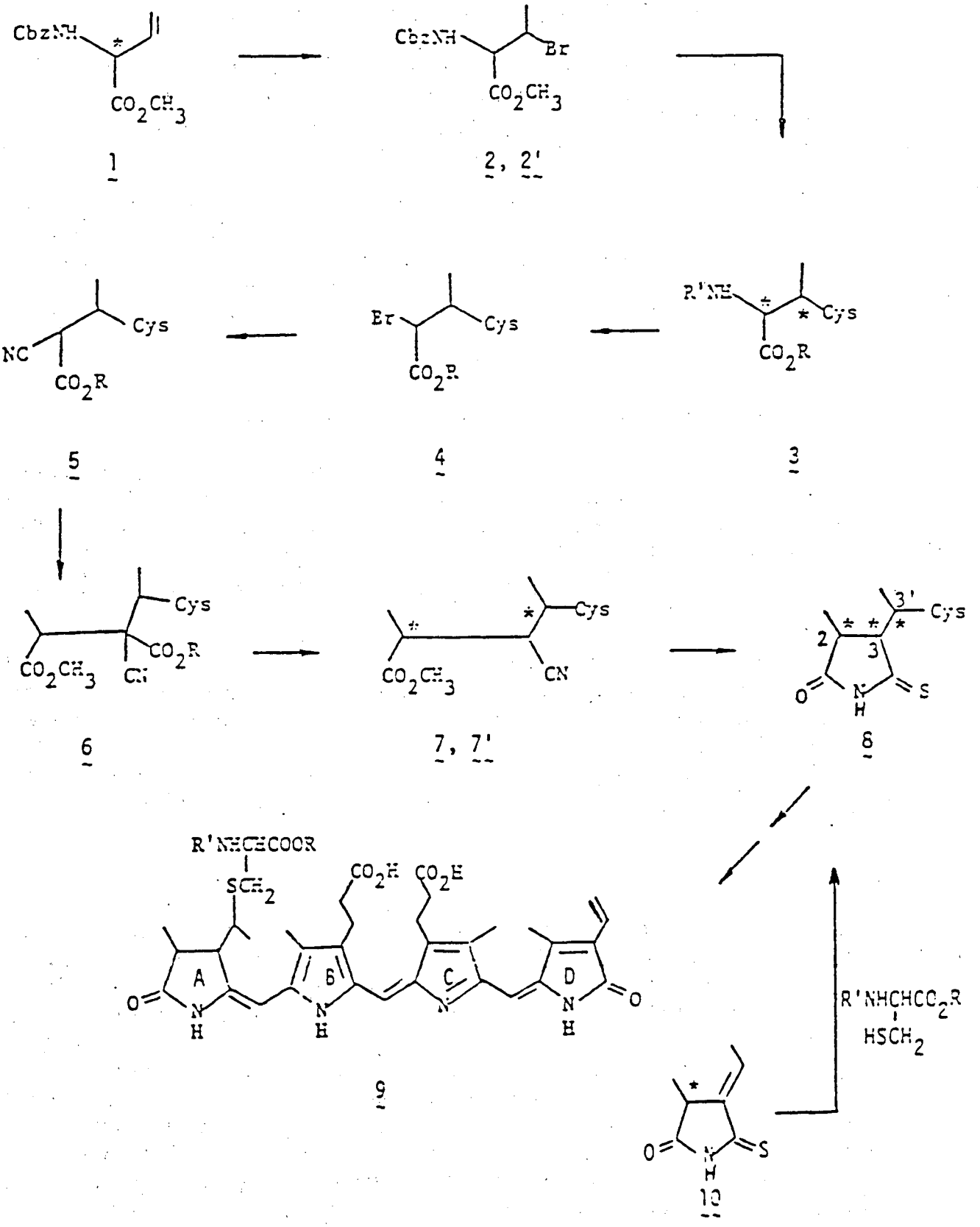

CbzNH
CO2CH3
1
CbzNH
Br
CO2CH3
2, 2'
R'NH
Cys
CO2R
3
Br
Cys
CO2R
4
NC
Cys
CO2R
5
Cys
CO2R
CO2CH3
CN
6
Cys
CO2CH3
CN
7, 7'
3'
Cys
2
3
O
N
H
S
8
R'NHCHCOOR
SCH2
CO2H
CO2H
A
B
C
D
9
R'NHCHCO2R
HSCH2
10

Figure 1

6. HYDROCARBONS FROM PLANTS (M. Calvin and J. W. Otvos)

The overall goal of this program is to develop an understanding of the processes involved in hydrocarbon production in plants. Specifically, we are interested in the mechanisms and control of isoprenoid (terpenoid) biosynthesis. Acquiring this basic information will be necessary before we can genetically manipulate plants to increase hydrocarbon yields.

We use the latex isolated from laticifer cells of the *Euphorbia lathyris* plant for our biosynthetic studies. These cells are the site of both the biosynthesis and the storage of large quantities of sterols (triterpenols). We are currently examining the final steps of sterol synthesis, the epoxidation and cyclization of squalene, to determine if more than one squalene cyclase is involved in sterol synthesis. We are also attempting to identify the organelle involved in the conversion of mevalonic acid (MVA) to sterols.

Environmental Effects on Hydrocarbon Production (Latex Biosynthesis)

It may be possible to increase the hydrocarbon content of plants by changing the environment around these plants. We have found that by increasing the day length from about 12 hours to 16 hours and by maintaining a constant day/night temperature region, we observed about a 9-fold increase in the activity of 3-hydrox-3-methyglutaryl-Coenzyme A Reductase (HMGR). Since this enzyme plays a key role in the synthesis of isoprenoids, it is possible that hydrocarbon production is sensitive to environmental controls.

Controlled changes in environmental conditions could restrict growth while having only small effects on the photosynthetic rate (per area). If applied when the plant approached maturity, this could result in an increase in assimilates available for partitioning into isoprenoid biosynthesis. We also hope to identify the internal controls of carbon allocation, since they also could be manipulated to increase plant hydrocarbon production. To investigate these possiblities we are observing the effects of salinity stress and water stress on hydrocarbon production.

Stress Studies

After establishing the growth conditions necessary to grow *E. lathyris* hydroponically, we completed the preliminary study of salinity stress and water stress. The effects of salinity on growth were determined from changes in shoot length, total fresh weight and root and shoot dry weights. Changes in the photosynthetic apparatus were determined from chlorophyll content, thylakoid proton gradient formation, and *in vivo* fluorescnece patterns. Changes in the levels of energy-rich compounds were determined by heptane and methanol extractions of the dried plant. Only the shoot portion of the plant was used for this extraction as this is what would be available for harvest. The results indicate that salinities of 50 mM NaCl and greater affect both growth and photosynthesis, though the reduction in growth is sharper than the reduction in photosynthesis. Results of extraction experiments with plant components also indicate that increased salinity causes an increase in carbon allocation to the heptane and methanol fractions, with most of the increase occurring in the carbohydrate-rich methanol fraction. Salinization also

caused a 3-fold increase in HMGR activity. Water stress was also found to increase carbon allocation to hydrocarbon production.

Regulation of Terpenoid Production in Euphorbia

The regulatory enzyme HMGR has been associated with various organelles in higher plants. Its localization in latex is not yet known, but our results indicate that it is associated with an as yet unidentified organelle. We are studying latex organization not only for the purpose of locating HMGR, but also to investigate other aspects of isoprenoid synthesis. We are already able to separate the site of (MVA) to triterpene (C_{30}) synthesis from the site of HMGR activity. The site of MVA metabolism is also an organelle, but different from the site of HMG-CoA metabolism. By separating the two sites we now have a system where we can study some of the latex stages of isoprenoid production in latex.

Organization of Euphorbia Latex.

Previous differential centrifugation studies have indicated that the synthesis of sterols from acetate can be physically divided into two separate processes: The conversion of acetate to MVA and then the conversion of MVA to sterols. These results also indicate that the two processes are compartmentalized in two separate subcellular organelles. Since compartmentalization is one form of biological control, we have attempted to isolate the individual organelles found in *E. lathyris* latex, so that we may study further their role in the control of isoprenoid biosynthesis.

We have localized specific organelles in latex using differential and Percoll gradient isopycnic centrifugation, marker enzymes and electron microscopy. Enzymes specific for mitochondria, endoplasmic reticulum (microsomes) and vacuoles all have been found in latex. Tests for peroxisomes and rough endoplasmic reticulum have provided negative results. We have identified the particle responsible for the conversion of MVA to sterols as a vacuole.

Mechanism of Squalene Cyclization

The major components of *E. lathyris* latex are tetracyclic triterpenoids, which are stored as free triterpenols, and their fatty acid esters. To understand the biosynthetic processes involved in the production of sterols it is necessary to elucidate the structures of these compounds. We have previously identified four of the six major triterpenols as cyloartenol, 24-methylenecycloartenol, lanosterol and 24-methylenelanosterol. With the results obtained from ^{3}H-NMR, chemical shift reagents, ^{13}C-NMR and optical rotation we have identified the fifth compound as butyrospernol.

We have made a preliminary identification of the sixth compound as euphol on the basis of its behavior in gas chromatography.

In most plant systems the initial product of squalene cyclization is cycloartenol and not lanosterol as found in animals and fungi. However, since lanosterol is a major component of *E. lathyris*

latex, we are attempting to determine if lanosterol is (a) a product of cycloartenol, (b) produced via separate, parallel pathway, or (c) is a precursor of cycloartenol. To determine if one of the first two mechanisms is responsible for squalene cyclization, we have synthesized deuterium-labeled MVA to be used as a susbtrate to follow its latex-catalyzed conversion to lanosterol. We are currently using radiolabelled precursors to elucidate the pathway of squalene cyclization.

PUBLICATIONS – BIOLGICAL ENERGY RESEARCH

1. Bassham, J. A., and Taylor, S. E. Carbon Metabolism. In Methods in Enzymology: Cyanobacteria (L. Packer, AN Glazer, eds) Academic Press, N.Y. In Press.
2. R.D. Britt, K. Sauer, and M.P. Klein. Electron Spin Echo Studies of PS II Membranes. **In**: Progress in Photosynthesis Research, vol. I (J. Biggins, Ed.) Martinus Nijhoff Publ., Dordrecht, Netherlands (1987), pp. 573-576.
3. R. David Britt and Melvin P. Klein. A Versatile Loop–Gap Resonator Probe for Low Temperature Electron Spin Echo Studies. J. Mag. Res. (in press)
4. R.H. Boutin and H. Rapoport. α-Amino Acid Derivatives as Chiral Educts for Asymmetric Products. Synthesis of Sphingosine from α'-Amina-α,β-Ynones. J. Org. Chem. 51, 5320 (1986).
5. T.F. Buckley and H. Rapoport. U.S. Patent 4,618,710 (to Regents, U.C.) Oct. 21, 1986.
6. M. Calvin. Renewable Fuels and Materials: Oil from Plants. Cell Biophys. **9**, 189 (1986).
7. Calvin, M., High Energy Fuels and Materials from Plants. J. Chem. Educ. **64** 335 (1987).
8. Calvin, M. Fuel Oils from Euphorbs and other Plants. Bot Jour. Linnean Soc., **94** 97 (1987)..
9. J. Cole, V. K. Yachandra, R. D. Guiles, A. E. McDermott, R. D. Britt, S. L. Dexheimer, K. Sauer and M. P. Klein. Assignment of the g=4.1 EPR Signal to Manganese in the S_2 State of the Photosynthetic Oxygen-Evolving Complex: An X- ray Absorption Edge Spectroscopy Study, Biochim. Biophys. Acta **890**, 395-398 (1987).
10. J. Cole and K. Sauer. The Flash Number Dependence of EPR Signal II Decay as a Probe for Charge Accumulation in Photosystem II. Biochim. Biophys. Acta **891**, 40-48 (1987).
11. J. Cole and K. Sauer. The Flash Number Dependence of EPR Signal II Decay as a Probe for Charge Accumulation in Photosystem II. **In**: Progress in Photosynthesis Research, vol. I (J. Biggins, Ed.). Martinus Nijhoff Publ., Dordrecht, Netherlands, (1987) pp. 569-572.
12. J. Cole, V.K. Yachandra, R.D. Guiles, A.E. McDermott, R.D. Britt, S.L. Dexheimer, K. Sauer and M.P. Klein. Assignment of the g=4.1 EPR Signal to Manganese in the S_2 State of the Photosynthetic Oxygen-Evolving Complex: An X-Ray Absorption Edge Spectroscopy Study. Biochim. Biophys. Acta **890**, 395-398 (1987).
13. J. L. Cole, Vittal K. Yachandra, Ann E. McDermott, R.D. Guiles, R.D. Britt, S.L. Dexheimer, Kenneth Sauer and Melvin P. Klein. Structure of the Mn Complex of Photosystem II upon Removal of the 33 kDa Extrinsic Protein: An X-ray Absorption Spectroscopy Study. Biochem. (in press).

14. P.L. Feldman and H. Rapoport. Synthesis of Optically Pure Δ^4-Tetrahydroquinolinic Acids and Hexahydroindolo[2,3-a]quinolizines from L-Aspartic Acid. Racemization on the Route to Vindoline. J. Org. Chem. **51**, 3882 (1986).

15. R.D. Guiles, V.K. Yachandra, A. McDermott, R.D. Britt, S.L. Dexheimer, K. Sauer, and M.P. Klein. Structural Features of the Manganese Cluster in Different States of the Oxygen Evolving Complex of Photoystem II: An X-ray Absorption Spectroscopy Study. **In**: Progress in Photosynthesis Research, vol. I (J. Biggins, Ed.). Martinus Nijhoff Publ., Dordrecht, Netherlands (1987), pp 561-564.

16. Hawkins, D. R., and Calvin, M. A Simple Efficient Synthesis of 3'-$^2H_3$-Mevalonic Acid. J. Labelled Cmpds. In Press.

17. W.D. Lubell. Configurational Stability of N-Protected α-Amino Aldehydes. J. Am. Chem. Soc. **109**, 236 (1987).

18. A.E. McDermott, V.K. Yachandra, R.D. Guiles, R.D. Britt, R.D., S.L. Dexheimer, K. Sauer and M.P. Klein. Iron X–ray Absorption Spectroscopy of Low Potential Acceptors in Photosystem 1.Progress in Photosynthesis Research vol. 1, p. 249–252 (1986).

19. A.E. McDermott, R.D. Guiles, V.K. Yachandra, J. Cole, R.D. Britt, S.L. Dexheimer, K. Sauer, and M.P. Klein. X-Ray Absorption Spectroscopy of Manganese and Iron in Photosynthetic Apparatus. Proc. International Conference on Biophysics and Synchrotron Radiation, Frascati, Italy, July 1986, Springer-Verlag, Berlin, in press.

20. A.E. McDermott, V.K. Yachandra, R.D. Guiles, R.D. Britt, S.L. Dexheimer, K. Sauer and M.P. Klein. Characterization of the Mn–Containing O_2–Evolving Complex from the Cyanoabacterium *Synechococcus* Using EPR and X–Ray Absorption Spectroscopy. Progress in Photosynthesis Research Vol. 1, p. 565-568 (1987).

21. A.E. McDermott, V.R. Yachandra, R.D. Guiles, R.D. Britt, S.L. Dexheimer, K. Sauer, and M.P. Klein. Iron-X-Ray Absorption Spectra of Acceptors in PS I. **In**: Progress in Photosynthesis Research, vol. I (J. Biggins, Ed.). Martinus Nijhoff Publ., Dordrecht, Netherlands (1987).

22. A. McDermott, V.K. Yachandra, R.D. Guiles, R.D. Britt, S.L. Dexheimer, K. Sauer, and M.P. Klein. Characterization of the Mn-Containing O_2-Evolving Complex from the Cyanobacterium *Synechococcus* using EPR and X-Ray Absorption Spectroscopy. **In** Progress in Photosynthesis Research, vol. I (J. Biggins, Ed.). Martinus Nijhoff Publ., Dordrecht, Netherlands (1987).

23. A. E. McDermott, V. K. Yachandra, R. D. Guiles, R. D. Britt, S. L. Dexheimer, K. Sauer and M. P. Klein. The States of Iron and Manganese in the Photosynthetic Apparatus Determined by X-Ray Spectroscopy. Proc. International Conf. on Biophysics and Synchroton Radiation. A. Bianconi (ed) Springer-Verlag. (in press)

24. M.P. Moyer, J.F. Shiurba and H. Rapoport. Metal-Halogen Exchange of Bromoindoles. A Route to Substituted Indoles. J. Org. Chem. **51**, 5106 (1986).

25. Otvos, J. Energy Content of Biomass: Calculations from Elemental Composition. Biomass, In Press

26. K. Sauer. Photosynthetic Light Reactions - Physical Aspects. **In**: Encyclopedia of Plant Physiology [new series], vol. 19, Photosynthetic Membranes (L.A. Staehelin and C.J. Arntzen, Eds.). Springer-Verlag, Berlin, (1986) pp. 85-97.

27. K. Sauer and H. Scheer. Fluorescence Decay and Depolarization Kinetics Calculated Using Förster Inductive Resonance and the Molecular Coordinates for C-Phycocyanin. **In**: Progress in Photosynthesis Research, vol. I (J. Biggins, Ed.). Martinus Nijhoff Publ., Dordrecht, Netherlands, (1987) pp. 139-142.

28. K. Sauer, H. Scheer, and P. Sauer. Excitation Transfer Rates in C-Phycocyanin. Förster Transfer Calculations Based on Crystal Structure Data from *Agmenellum quadruplicatum* C-Phycocyanin. Photochem. Photobiol., in press.

29. Skrukrud, C. L., Taylor, S. E., Hawkins, D. R., and Calvin, M. Triterpenoid Biosynthesis in *Euphorbia lathyris*. In The Metabolism, Structure and Function of Plant Lipids (PK Stumpf, JB Mud, WD Nes, eds) Plenum Press, N. Y. pp. 115–119 (1987).

30. Taylor, S. E., and Calvin, M. Hydrocarbons from Plants: Biosynthesis and Utilization. Comments Agric. & Food Chem. **1** 1 (1987).

31. Taylor, S. E., Skrukrud, C. L., and Calvin, M. The Effect of Salinity on the Allocation of Carbon to Energy–rich Compounds in *Euphorbia lathyris*. In Energy from Biomass and Wastes (D. Klass, ed) IGT, Chicago, In Press.

32. S.T. Worland, A. Yamagishi, S. Isaac, K. Sauer, and J.E. Hearst. Labelling Quinone Binding Sites in Photosynthetic Reaction Centers: A 38 Kilodalton Protein Associated with the Acceptor Side of Photosystem II. Proc. Nat. Acad. Sci. USA **84**, 1774-1778 (1987).

33. V. K. Yachandra, R. D. Guiles, A. McDermott, R. D. Britt, S. L. Dexheimer, K. Sauer and M. P. Klein. The State of Manganese in the Photosynthetic Apparatus. 4. Structure of the Manganese Complex in Photosystem II Studied Using EXAFS Spectroscopy. The S_1 State of the O_2-Evolving Photosystem II Complex from Spinach. Biochim. Biophys. Acta. **850**, 324-332 (1986).

34. V. K. Yachandra, R. D. Guiles, K. Sauer and M. P. Klein. The State of Manganese in the Photosynthetic Apparatus. 5. The Chloride Effect in Photosynthetic Oxygen Evolution. Is Halide Coordinated to the EPR-active Manganese in the O_2-Evolving Complex? Studies of the Substructure of the Low- temperature Multiline EPR Signal. Biochim. Biophys. Acta **850**, 333-342 (1986).

35. V.K. Yachandra, R.D. Guiles, A. McDermott, J. Cole, R.D. Britt, S.L. Dexheimer, K. Sauer, and M.P. Klein. The State of Manganese in the Photosynthetic Apparatus: An X-Ray Absorption Spectroscopy Study. **In**: Progress in Photosynthesis Research, vol. I (J. Biggins, Ed.). Martinus Nijhoff Publ., Dordrecht, Netherlands (1987), pp. 557-560.

36. V. K. Yachandra, R. D. Guiles, Ann McDermott, R. D. Britt, J. Cole, S. L. Dexheimer, K. Sauer and M. P. Klein. The State of Manganese in the Photosynthetic Apparatus Determined by X-ray Absorption Spectroscopy. Proc. Int. Conf. on EXAFS and Near Edge Structure IV. (P. Lagarde and D. Raoux, eds.) J. de Physique Vol. 47, C8-1121-1128 (1987)

37. V. K. Yachandra, R.D. Guiles, A. E. McDermott, J.L. Cole, R. D. Britt, S.L. Dexheimer, K. Sauer and M.P. Klein. Comparison of the Structure of the Mn Complex in the S1 and S2

States of the Photosynthetic O_2-Evolving Complex: An X-Ray Absorption Spectroscopy Study. Biochemistry, in press.

38. Y.S. Zhu, D. Cook, F. Leach, G. Armstrong, M. Alberti, J.E. Hearst. Oxygen-Regulated mRNAs for the Light-Harvesting and Reaction-Center Complexes and Bacteriochlorophyll and Carotenoid Biosynthesis in *Rhodobacter capsulatus* during the Shift from Anaerobic to Aerobic Growth. J. Bacterio. **168**(3), 1180-1188 (1986).
39. Y.S. Zhu and J.E. Hearst. Regulation of Expression of Genes for Light Harvesting (LH-I, LH-II), Reaction Center (RC-L,M,H), Bacteriochlorophyll and Carotenoid Biosynthesis in *Rhodopseudomonas capsulata* by Light and Oxygen. Proc. Nat. Acad. Sci. U.S.A. **83**, 7613-7617 (1986).
40. Y.S. Zhu and J.E. Hearst. Oxygen and Light Regulation of Expression of Genes for Light Harvesting (LH-I, LH-II), Reaction Center (RC-L, RC-M, RC-H), Pigment Biosynthesis and a Transcriptional Role in the Protective Function of Carotenoids in *Rhodobacter capsulatus*. Proceedings of the VII International Congress of Photosynthesis, ed. Biggins, 717-720 (1987).

HAO2 – ENVIRONMENTAL RESEARCH AND DEVELOPMENT

The Chemical Biodynamics programs in the Energy Research and Development section of DOE involve understanding the relationship between nucleic acid structure and function.

- The studies include determining the stucture of protein-DNA complexes by X-ray crystallography, NMR, ^{3}H-NMR, and fluorescence correlations spectroscopy. This work is aimed at determining how these two types of macromolecules find each other and how agents which cause mutations perturb their interactions.

- This program contains work which is directed at understanding the mechanism of *rec A* catalyzed recombination. This project utilizes the expertise in our laboratory with psoralen crosslinking of nucleic acids to trap intermediates in the recombination process.

- We are studying the regulation of replication of extrachromosomal DNA in human cells. We are determing how structural modifications in the origin–promoter–enhancer region of these DNA'es effect the replication efficiency in various cell types. These studies will help us understand how the initiation of DNA synthesis is regulated in various cell types, and may also help us design new DNA vectors for human cells.

Underlying all of these studies is a continuing effort to understand the basic structure of nucleic acids and how secondary structures within the molecules regulation functions of genes.

1. BIOMOLECULAR STRUCTURE ANALYSIS BY NMR – Professor David E. Wemmer

Our efforts this year have been directed to several problems of protein and oligonucleotide structure, and have used high resolution NMR spectroscopy extensively. The general goals of the projects described are: to understand the solution structure of DNA molecules with a nick or gap in the sugar phosphate backbone as simple models for damaged DNA; to determine the structure of a drug/DNA complex, and determine the microscopic kinetics of the binding steps; and to analyse the structures of several small cystine rich proteins to enhance our understanding of the active parts of the structure. A significant amount of time has gone into implementation of methods for preparing large quantities of secific sequence DNA oligomers for study, and to setting up the necessary software on our VAX computer systems for analysis of NMR data, and the required structure calculations. The results of these efforts are discussed for particular projects below.

Gap DNA structures

By choosing special sequences for synthetic DNA oligomers we have been able to form stable "sticky end" dimers in solution from DNA hairpin with dangling ends. The sequences are chosen with a central section which is not complementary to any other in the molecule, which is flanked by a self-complementary sequence. Such sequences have been shown previously to lead to hairpin formation. We have modified these sequences to include a self complementary dangling tail, which allows two such molecules to associate in solution. We have observed the imino proton spectra from these molecules to confirm that such a dimer is indeed the stable form in solution. The imino protons have been assigned using nuclear Overhauser effect difference spectra, and confirm that the dimer is essentially a single continuous double helix in solution. On each of the backbone strands, however, there is a break and a missing phosphate. We have now collected complete two dimensional NOE data sets (NOESY spectra) of two different sequences. In these spectra, when collected at sufficiently short mixing times, the cross peak intensities reflect the inverse sixth power of distances between protons. By analysing the intensities of many cross peaks in the spectrum, and paying particular attention to those at the site of the gap in the backbone, we have determined that the backbone is relatively undistorted, as compared to a standard "B DNA" even at the site opposite the gap. There does appear to be a slight overwinding of the DNA at this site, giving a slightly greater than average local helix twist angle. We have also used the temperature dependence of the NMR spectrum to examine the melting in these sequences. We find that, as expected, melting occurs first as a separation of the dimer into monomers, followed at higher temperature by opening of the hairpin loop. For sequences with 4 GC base pairs in the overlap region, and six in the stem of the hairpin loop these melting events are separated by about 15°C, with the first transition having slower exchange kinetics (between monomer and dimer) than the latter.

DNA-drug binding

We have previously carried out NMR studies of the binding of the antibiotic distamycin-A to a specific DNA dodecamer. This drug shows a strong preference for binding at AT rich sequences,

AATT in the cases which we have studied in detail. From the NMR data it was possible to position the drug relative to the DNA, and to qualitatively evaluate the degree of distortion of the DNA by the drug. We have now supplemented these measurements by carrying out energy minimization calculations with the AMBER program from UCSF. A general problem with such calculations is that the minimization algorithm cannot find a global minimum of energy in a reasonable time. We find that starting with the drug relatively far from the position determined from NMR, convergence is obtained, but to a drug binding position which is far from that experimentally determined. However, when the calculation is started near the experimentally determined structure the energy obtained upon convergence is lower. The coordinates obtained through such experimentally guided modelling are better than what can be determined from the NMR data alone. Presently we are trying to extend the measurements to other DNA sequences, and to analyse the differences in binding constant and kinetics of drug binding in light of the detailed structural information we now have about the bound state.

Analysis of protein structures

We are interested in the determination of protein structures in solution for several reasons. First, we expect to be able to use cystines at specific residues in proteins to predictably fold the remainder of the sequence. The expectations are based upon the observed folding in several naturally occuring peptides, which have common cystine positions. While we have previously assigned the resonances in two of these peptides, and qualitatively modeled their secondary structure, it is important to carry out a more quanititative analysis. To do this we have collected 2D NOE spectra and integrated cross peak intensities to obtain interproton distances for apamin, a small neurotoxic peptide from honey bee venoCm. With a relatively large number of such distances we have carried out distance geometry calculations. This approach takes the distance estimates, including estimates for the experimental precision, and computes from these coordinates for structures which are consistant with all of the input data. From the range of structures obtained from repeated calculations we can analyse the precision with which the structure is determined, e.g. its effective "resolution".

In a similar fashion we have begun analysis of the relationship between structure, function and immunogenicity of protein toxins isolated from sea anemones. These again are related through having common cystine positions, and several other conserved residues. However different toxins from this family are active against different receptors, and do not all show antibody cross reactivity. We have now fully assigned resonances of the Radianthus paumotensis toxin II, and have established that its only regular secondary structure is beta sheet, with strands connected with a variety of loops and turns. We will now begin structure calculations for this protein, and at the same time have begun assigning a related protein Rp III. We have just obtained the sequence of Rp III through a collaboration with Prof. Ken Walsh at the University of Washington, and have already established that there is a high degree of homology in the structures. From the refined structures for proteins in a related family, such as these, we should be in a good position to look for the common structural features which give rise to the similar folding of the peptide chain, and yet be able to see the differences which lead to different activities, and immunogenicity.

2. BIOPHYSICAL CHEMISTRY – Dr. Melvin P. Klein

In Vivo NMR Spectroscopy

Phosphorus-31 NMR spectroscopy is evolving into an important means for determining the *in vivo* concentrations of phosphorylated metabolites and is now entering the clinical arena. Our previous contributions to this field demonstrated the feasibility of employing implanted radio frequency coils around organs of laboratory animals to permit eliciting the NMR spectra over long periods to establish normative spectra. Using these devices and techniques we have determined phosphorus exchange reactions in rat hearts and kidney, *in situ*, and have demonstrated that there are pools of metabolic intermediates that are not directly visible in the conventional high resolution NMR spectra. Comparison of the results from NMR spectroscopy with those obtained from radiolabeling studies on chick embryo fibroblasts also showed that there are significant pools of phosphorus not visible in the P-31 NMR spectrum. Both sets of studies suggest that compartmentation occurs. The invisibility of these pools is assumed to result from the immobilization of the molecules by cellular macromolecules or organelles.

The two principle mechanisms leading to broadening of the P-31 resonances are chemical shift anisotropy and dipole-dipole coupling with protons. The former may be removed by the technique of magic angle sample spinning (MASS) while the latter is reduced by application of strong decoupling fields applied at the resonance frequency of the protons. When appropriately applied the decoupling method also leads to significant signal enhancement from the cross polarization. Were it possible to subject either an animal or tissue to the MASS experiment, the P-31 spectrum would exhibit resonances from all of the phosphorus containing molecules, even those of the nucleic acids.

To provide the reference spectra to guide such tissue experiments we have obtained the MASS spectra for a large number of biological phosphorylated molecules including many for which crystal structure data are available. The confluence of the crystal structure and chemical shift tensor data has permitted us to establish a strong correlation between the values of the shift tensor elements and bond lengths/bond angles within the phosphate moiety. These results in turn have permitted us to test the validity of some theoretical predictions for the origins of P-31 isotropic chemical shifts.

Of direct biological importance has been the determination of the chemical shielding tensors for crystalline Mg:ATP, Ca:ATP and Na:ATP. It is well established that the isotropic chemical shifts observed in solution spectra for the ATP molecules are sensitive to metal complexation and to pH. Our measurements in the solid state provide the rationale for such observations. In the Mg and Ca-ATP forms the terminal or gamma phosphates behave as mono-esters. By contrast, the terminal phosphate in Na-ATP behaves as a di-ester because it is protonated.

Technical limitations have precluded thus far the application of these techniques to intact tissue samples. We have, however, used lyophylized tissue obtained by surgical freeze-clamp excision. The P-31 MASS spectra of such tissue exhibit features assignable to the nucleic acids, to phospholipid phosphate and to ATP. That ATP is observed is confirmation that the tissue was

excised in the energized state. Not suprisingly there is a larger content of ATP in muscle tissue than in either liver or kidney. The ATP features are attributable to the Mg or Ca form rather than the free or protonated form thus resolving a long standing question in bioenergetics.

The proton spectra of the lyophylized tissue subject to MASS exhibit a rich spectrum of very narrow lines reminiscent of solution spectra. Our prior extensive work on membranes and model membrane systems provided the background to recognize that these signals originated from the fatty acid chains of the membrane phospholipids in the tissue. Resonances can be assigned to protons ranging from the glycerol group to many moieties in the acyl chain including the unsaturated carbons and the terminal methyls. The N-methyls of the choline headgroups are virtually invisible.

We are able to simulate the P-31 MASS spectra by including the solid phosphorus features of nucleic acids, phospholipids and mono- and di-esters in appropriate quantities. These simulations show that the phospholipid head groups are immobile. Simulations of the nucleic acid phosphorus demonstrate some hydration, to the extent of perhaps 10 molecules of water per base pair.

The presence of high resolution features from the fatty acid chains demonstrates that they are executing relatively rapid reorientational motions: the absence of signals from the choline N-methyls, together with the broad phospholipid phosphorus resonances, implies that the motion of the headgroups is severely limited. In summary, then, these findings are interpreted to indicate that, at the temperature of observation, the phospholipids are in the liquid crystal state characteristic of their composition and are executing the dynamics associated with their phase diagram. Normal functioning of the cellular membrane, as exemplified by the fluid-mosaic model, is assumed to require a high deree of dynamic mobility. That we observe such high resolution proton spectra in lyophylized tissue is indeed dramatic support for such a model.

The Chemical Biodynamics Division of LBL has inaugurated Tritium NMR (TMR) spectroscopy in conjnunction with the establishment of the National Tritium labeling Facility. The potential applicatiions of TMR to problems of structural biology and biophysics are very great. They promise to extend the molecular weight range of molecules that can be profitably studied with NMR by several fold, will permit the study of interactions between enzymes and bound substrates, between receptors and effectors, and between proteins and nucleic acids. This potential derives from the facts that the intrinsic sensitivity of the triton is some 7% greater than that of the proton, that there will be zero interferring background signals and because the tritium spectrum will be sparse, arising only from those tritons at the sites specifically labeled. Importantly, the abundant protons can be decoupled from the tritons thus reducing their contributions to resonance broadening.

During June of 1986 a new 300 MHz NMR spectrometer specifically configured for optimum utility with TMR was installed in the laboratory. The sensitivity anticipated and realized demonstrates that we shall be able to work with samples containing millicuries or less tritium.

In a first application of TMR to a biological problem, we have observed the conversion of glucose, tritiated at the C-1 position, to lactate by mammalian erythrocytes. During this metabolism we

observe that the two anomers of glucose are metabolized at different rates. Several intermediates are observed. The major component has been identified as 2,3 diphosphoglycerate. Upon completion, the spectrum consists of the lactate methyl resonances and some tritiated water. This water or OT resonance is at the submicromolar level and provides a method for observing directly some hitherto unobserved pathways of hydrogen in this important metabolic system. The kinetics of the individual steps in the reaction have been determined directly from these *in vivo* observations. Heretofore it was necessary to analyze extracts of cell

---

3. MUTAGENESIS - FUNDAMENTAL CHEMISTRY – Professor John E. Hearst

Study of the Action Mechanism of ABC Exinuclease on Psoralen Modified DNA Substrates

ABC excinuclease is an ATP-dependent DNA repair enzyme that removes nucleotides modified (damaged) by a vast array of carcinogens (see Friedberg, 1985 for a review). The enzyme is made up of three subunits, UvrA (103,873 Daltons), UvrB (76,118) and UvrC (66,038) and cuts the damaged DNA strand on both sides of the modified nucleotide(s). Using unique DNA sequences that have been modified randomly by UV (254 nm) irradiation, psoralen plus near UV (360 nm), cisplatin, and acetylaminofluorene as substrates, extensive data has been accumulated that is consistent with cutting by the enzyme of the 8th phosphodiester bond 5' and the 4th or 5th phosphodiester bond 3' to diadducts and the 8th (5') and the 5th (3') phosphodiester bonds on the two sides of nucleotide monoadducts (Sancar and Rupp, 1983; Sancar et al., 1985; Beck et al., 1985). Although all existing data are consistent with this cutting pattern a number of anomalies have been observed (Sancar and Rupp, 1983; Yeung et al., 1983; Sancar et al., 1985). Therefore, is has been desirable to have a uniquely modified DNA for a precise definition of the cutting pattern of the enzyme. The construction of such psoralen containing substrates has enabled us to determine the cutting site of ABC excinuclease with regard to two types of HMT monoadducts and a HMT interstrand diadduct (crosslink), as well as to study the interaction of ABC excinuclease with DNA by DNAse I footprinting. The preliminary footprinting data show that binding of the A and B subunits to the substrate, while apparently protecting the entire 40 bp fragment, induces two phosphodiester bonds 5' to the damaged nucleotide to become hypersensitive to DNAse I cleavage, suggesting that the UV-induced single strand breaks observed in uvrC minus mutants (see Tang and Ross, 1985) result from non-specific incision of these hypersensitized bonds by any of the many *E. coli* endonucleases.

Analysis of the Kinetics and Structure of the Three–Strand Complex of recA

Two distinct lines of thought motivate interest in this study of the recA protein from *E. coli* and its interactions with DNA. First, such studies can contribute to the understanding of the mechanisms involved in general recombination through an understanding of recA's actions. Secondly, a protein such as recA may facilitate the *in vitro* hybridization of short oligomeric probes to target sequences within long (kbpr) substrates through its ability to promote homologous pairing.

RecA is actually a multifunctional enzyme. As an ATPase and helicase-recombinase, recA

catalyzes DNA-dependent hydrolysis of ATP and ATP-dependent pairing of DNA, either of complementary single strands or of single-stranded (ss) with homologous double-stranded (ds) DNA. As a protease, the enzyme is responsible for the ATP- and polynucleotide-dependent proteolysis of certain regulatory proteins. These include the LexA protein, a multioperon repressor which acts upon the recA gene itself. Through its recombinatory actions, then, the recA protein has a role in postreplication repair. Its protease functions are associated with the SOS response, a series of metabolic changes initiated within cells whose DNA has been damaged. (Reviewed by McEntee and Weinstock, 1981).

Homologous pairing directed by recA may be separated into three stages (Radding et al., 1982). Initially, recA cooperatively polymerizes along ssDNA to form a presynaptic complex. In synapsis, the complexed ssDNA is first brought into conjunction and then homologous alignment with duplex DNA. Two distinct structures have been noted in this process of homologous alignment: First to form is a paranemic joint in which the complementary strands are aligned, but without any net interwinding; a plectonemic joint, the interwound complex, forms next. Clearly, substrate topology is a consideration in the formation of this latter joint. The postsynaptic stage involves unidirectional branch migration resulting in extended heteroduplex complexes (D-loops) or complete strand exchange. If the plectonemic joint has formed at the 3'end of the incoming single strand, the duplex DNA will be unwound by 5*I*-β-3*I* branch migration along the complementary strand, and the three-stranded complex will dissociate (Wu et al., 1982).

The proposed approach to study recA-DNA complexes is to utilize psoralen crosslinkages to freeze the heteroduplexes upon formation, preventing displacement of the inserted strand due to reannealing of the parent duplex. This is critical if oligomeric probes are to be used. Because crosslink formation is a photochemical reaction, time course studies are possible, and equilibrium data is within reach. If either recA or uvsX, its analog from phage T4, are observed to enhance hybridization and crosslinking efficiency, applications to psoralen probe technology may be possible.

References

1. Beck, D., Popoff, S., Sancar, A. and Rupp, W. D. (1985) Nucl. Acids Res. 13, 7395-7412
2. McEntee, K. and Weinstock, G. M. (1981) The Enzymes, P. Boyer, ed., Vol 14 445-470
3. Radding, C. M. (1982) Ann. Rev. Genet. 16, 405-437
4. Sancar, A. and Rupp, W. D. (1983) Cell, 249-260
5. Sancar, A., Franklin, K. A., Sancar, G. B. and Tang, M. S. (1985) J. Mol. Biol. 184, 725-734
6. Tang, M. S. and Ross, L. (1985) J. Bacteriol. 161, 933-937
7. Wu, A. M., Kahn, R. DasGupta, C. and Radding, C. M. (1982) Cell 30, 37-44
8. Yeung, A. T., Mattes, W., Oh, E. and Grossman, L. (1983) Proc. Nat. Acad. Sci. USA 79, 5470-5474

4. CONTROL OF DNA SYNTHESIS IN HUMAN CELLS – Dr. James C. Bartholomew

The genome of human cells contains approximately 10^9 nucleotide pairs organized into a particular sequence. The faithful replication of this amount of information into each daughter cells is obviously a formidable task. At each round of replication not only must the sequence of the DNA be preserved, but also the complement of genetic information must be maintained. A great deal of work has focussed on the mutational aspects of sequence changes, but little is known about the stability of the genome. A clue as to how the genome sequence is normally maintained in such a highly organized state during DNA replication is to learn something about the factors that destabilize the replication of the genome. Our research program centers on the hypothesis that much of the control of genome stability takes place at the level of initiation of DNA replication within sections of the genome. If initiation of DNA synthesis in a section of the genome occurs more than once per cell cycle, the extra copies of this section are available for gene amplification and/or rearrangement. We are interested in understanding what cellular factors regulate this initiation, and how external environmental stresses modulates the initiation of DNA synthesis at these sites.

Because the human genome is so large and the mechanisms regulating its replication likely to be so complex, we have attempted to develop model systems which can help us understand the control of initiation of DNA synthesis. For this reason we have been studying the replication of extrachromosomal DNA elements in human cells.

Extrachromosomal DNA elements have been observed by many investigators in both normal and transformed human cells. In general, these extrachromosomal elements are heterogeneous in sequence and contain a higher level of reiterated sequences than the total genome. Little is know about the function (if any) of these elements. They may be intermediates in recombinational events, genes in the process of being amplified, or residuals from the normal process of DNA replication. We have been studying the control of the replication of an extrachromosomal DNA element that has arisen in human cells transformed with SV40 virus. We have shown that these elements are unique in that they are derivatives of the wild type virus, replicate to high copy number in human cells, and yet maintain a stable DNA sequence without lysing the cells. We are trying to understand the factors that control the replication of these extrachromosomal elements with the hope that this understanding will tell us something about how human cells normally control the replication of genomic DNA.

Our studies began with the observation that two cell lines we obtained from the American Type Tissue Culture collection and which were transformed with wild type SV40 virus contained many copies of extrachromosomal DNA elements. The cell lines which harbor these extrachromosomal DNA'es are GM637, a human fibroblast cell line from an apparently normal individual, and XP12RO, a line from a *Xeroderma pigmentosum* patient containing a mutation in a DNA repair gene. Both of these cell lines were transformed with wild type SV40 by other investigators. The aim of these initial studies was to determine the involvement of DNA repair processes in gene amplification. These extrachromosomal DNA'es are derivatives of the input SV40 virus; however, they have undergone numerous sequence changes. Although human cells are known to be semipermissive for SV40 virus production, these DNA'es are unique in that they are mutated

and the mutation is preserved faithfully in the pool of DNA.

The origin of this extrachromosomal DNA and its relationship to the input transforming DNA is not known. To understand the system better we began to characterize the DNA. We found that in an actively growing population of GM637 cells the copy number of SV40 DNA is greater than 10,000 per cell. The extrachromosomal DNA is also present in XP12RO cells. On agarose gel electrophoresis, the extrachromosomal DNA from both cell lines has a lower molecular weight than wild type DNA. Restriction enzyme digestion indicates that the lower molecular weight is the result of a deletion in the A-gene coding for the T-antigen. In both cell lines the deletion maps near the boundary of the intervening sequence and the small exon of the T-antigen and removes 323 bp. The DNA from the XP12RO cell lines also contains an insertion of 18 bp in the large exon of the A-gene. DNA sequencing indicates that the deletion has eliminated one of the splice sites for the intervening sequence and has stopped just short of the translation termination site for the small t-antigen. We are presently studying the nature of the mRNA made from this deleted message. Our data indicates that the message is spliced at the normal small t-antigen splice sites, and so the gene may encode a protein of nearly the same size as the wild type large T-antigen. Its curious that two cell types that originated from separate transformation events give rise to very similar extrachromosomal deleted DNA.

We have also sequenced the orgin–promoter–enhancer region of the extrachromosomal DNA from the GM637 and XP12RO cells. In the DNA from both cell types, the origin region is the same as the wild type DNA origin. The enhancer region, however, in both DNA'es contains many rearrangements. Although the two DNA'es are different in this region, the deletions and duplications that occur lead to a similar overall structure. Since the enhancer region of the DNA is the site at which cellular factors interact to control the expression of the encoded genes, and in other systems, the host range of viral DNA replication, we suspect that it is these alterations in the enhancer region, possibly in conjunction with the alterations in the T-antigen caused by the deletion, gives rise to a stable replication of the DNA extrachromosomally in the human cells. Since the extrachromosomal DNA contains more than one type of mutation we are separating the mutations and placing them back individually into a wild type background to test which mutations are causing the altered phenotype. We are also beginning to do *in vitro* binding studies with wild type and mutated enhancer regions looking for differences in the way factors from the human cells bind to the different enhancer regions. It may be possible using this system to identify human cell factors responsible for the maintainance of the DNA as extrachromosomal elements.

The replication behavior of these DNA'es when introducted back into their normal host has been tested. The DNA from the human cells replicates with an efficiency less than the wild type DNA in CV-1 cells – a non-tranformed african green monkey kidney cell line. Furthermore, the DNA from the human cells failed to lyse the CV-1 cells. When the DNA'es were tranfected into a derivative of CV-1 that had a wild type T-antigen gene integrated into its genome, the replication efficiency of the DNA from the human cells was restored as well as the ability to express late gene functions.

It should also be noted that the extrachromosomal elements we are studying may serve as vehicles to introduced foreign DNA into human cells. The DNA appears to maintain a high copy number

in the human cells from which it was derived with very little rearrangement occurring in the DNA. We are presently reintroducing this DNA into human cells that have not previously been exposed to SV40 to see if the DNA will maintain itself as an extrachromosomal element. If these experiments are successful, we will clone marker genes into our DNA to see if that can be stably introduced and expressed in human cells.

---

5. STRUCTURAL BIOLOGY – Professor Sung-Hou Kim

Two major projects currently on going are:

(1) Structure determination of one of the most common human oncogene products, *ras* oncogene products (p21 proteins); and

(2) Structure determination of DNA damaged by a mutagen and UV radiation using NMR and x-ray crystallography.

Crystal Structures of *ras* Oncogene Products

There is compelling evidence that human cancer develops as a consequence of genetic changes (probably multiple) in some members of a selected set of cellular genes (Varmus, 1984). DNA isolated from a variety of tumors, but not normal tissues, possesses the ability to malignantly transform non-tumorigenic cells. Subsequently, many oncogenes responsible for such transformation have been isolated from human tumors and cell lines, and spontaneous, virus, chemical, or radiation-induced animal tumors.

The most commonly found oncogenes from human tumors and cell lines are the *ras* gene family (c-Ha-*ras*, c-Ki-*ras* and N-*ras*). The activation of *ras* genes is almost always acquired by point mutations which result in a single amino acid substitution at mostly either the 12th or 61st position of the coded protein, p21 (Tobin et al., 1982; Reddy et al., 1982; Per et al., 1986; for a review, see Levinson, 1986). These *ras* proteins are small (MW = 21,000; p21) and have been shown to be associated with the inner surface of the cell's plasma membrane (Willingham et al., 1980; Shih et al., 1980). All the *ras* p21 proteins have 189 amino acid residues and the amino acid sequence is very highly conserved except for the very end of the C terminal portion which also includes the membrane attachment site.

Ras genes have been found in a large number of eukaryotic organisms ranging from very low to very high eukaryotes. Such evolutionary conservation implies an important cellular function(s) for the normal *ras* genes. However the complete biological function of this protein is not known. Several biochemical properties, however, have been found to be associated witht the *ras* proteins: the proteins bind GDP and GTP; they hydrolyze GTP; they go through two post-translational modifications, i.e., acylation at position 186 and autophosphorylation at position 59 if threonine is at that position. Activated p21 proteins differ from their normal counterparts in their reduced GTP hydrolysis activity. It is necessary to add stearic acid to the cysteine residue at position 186 of p21 for it to have transforming activity probably because of its attachment to the cell membrane, although the amino acid sequence in the C-terminal region of p21 is not essential for

guanine nucleotide binding activity.

Among all the oncogene products, the *ras* oncogene product is best characterized biochemically. To obtain a structural basis for understanding the known biochemical functions of the proteins, to provide possible explanation for the exon/intron structure of the genes, and to search for the normal cellular function of the protein, the 3-dimensional structure of this family of proteins will be very useful. To achieve this goal, we have recently crystallized several c-H-*ras* p21 proteins which lack the C terminal heterogeneous region. The C terminal portion is not essential for the known biochemical function, except for its affinity to the membrane and its heterogeneity among the different forms of p21 proteins. Since it is known to be flexible, we have cloned a fragment coding for the residue 1-171 of the protein as well as the entire synthetic gene for a c-H-*ras* oncogene (K. Miura et al. [1986]). The synthetic gene was introduced into an *E. coli* vector containing the trp promoter of *E. coli* and a synthetic ribosome binding sequence.

Clones containing the genes were selected in an *E. coli* HB101 background and the DNA sequence of the insert was verified by dideoxy DNA sequencing methods (F. Sanger, 1981). The synthetic protein gene was expressed by induction with 3-indolyl-acrylic acid. The proteins were purified by DEAE Sephacel and Sephadex G-75 column chromatography to greater than 95% purity by essentially the same procedure as that reported by Gibbs et al. (1984). As detected by the complex formation with tritium labeled GDP, both truncated normal and activated proteins [p21(gly-12, 1-17) and p21 (val-12, 1-171)] bind GDP to the same extent as full-length p21s. The GTPase activity of p21 (gly-12, 1-171) was found to be the same as that of normal p21, whereas p21 (val-12, 1-171) showed, as predicted, almost no detectable GTPase activity.

We have crystallized an activated c-Ha-*ras* p21 protein fragment [p21 (val-12, 1-171)] from a solution containing 50 mM sodium Hepes buffer, pH 7.5, 0.1 mM $CaCl_2$, 1 mM Na-EDTA, 1 mM DTT and 0.01% n-octyl glucoside. The precipitating agent was 30% polyethylene glycol 400. These crystals proved to be quite stable upon exposure to X-rays and diffracted to a better than 2.5 Å resolution. The space group of the crystal form was $p6_122$ or $p6_522$ with unit cell parameters of a = b = 83.2 Å and c = 105.6 Å. There is one p21 fragment molecule per asymmetric unit and 46% of the unit cell volume was protein. We have also crystallized a normal c-Ha-*ras* p21 fragment [p21 (gly-12, 1-171)]. The cell parameters and space group were essentially the same as those of the activated c-Ha-*ras* p21 fragment crystals. The crystallized p21 fragments are likely to contain GDP. One possibility is that a substantial conformational change only occurs when p21 forms a complex with GTP. We have also recently crystallized a full-length normal c-Ha-*ras* protein [p21 (gly-12, gln-61, 1-189)] and an activated p21. X-ray analyses of these crystals is also being currently performed.

Structure of a Psoralen Cross–linked DNA in Solution Determined by Two–Dimensional NMR

Psoralens are a class of naturally occuring and synthetic furocoumarins (Cimino et al. 1985) that are used for treatment of psoriasis and other skin disorders (Parrish et al. 1974). In addition, psoralens have been used to inactivate viruses (Hearst and Thiry, 1977) and can cause

mutagenesis. They have also been used as *in vitro* and *in vivo* probes of nucleic acid structure and function because of their ability to covalently link nucleic acids by forming cross-links between opposing strands of duplex regions of DNA. A three-step mechanism of psoralen cross-linking with DNA has been proposed (Issacs et al., 1977). The planar psoralen first intercalates into a double helical region and initial UV irradiation (320-400 nm) induces a single cyclobutane addition with a pyrimidine base. The stereochemistry of the additional product between the 4'5' (furan) or 3,4 (pyrone) double bonds of psoralen and the 5,6 double bond of pyrimidine bases has been determined to be the cis-syn conformation (Kanne et. al., 1982). This has been confirmed by the three-dimensional structure of the monoadduct determined by X-ray crystallographic methods (Peckler et. al., 1982). Construction of physical models using this monoadduct structure led to the proposal (see last year's report) that psoralen cross-links caused a sharp kink in the double helical DNA structure and unwinding of the duplex (Kim et. al., 1983). A detailed molecular model of psoralen cross-linked DNA, using the monoadduct structure as the basis for computer model building and energy minimization calculations, has been presented (Pearlman et al., 1984).

Advances in high resolution two-dimensional NMR spectroscopy have provided us with a powerful tool for analyzing the solution structure of biological macromolecules (Wurtrich, 1984). The techniques of 2D NMR, using both through-bond couplings (COSY) and through-space couplings (NOESY), were applied to the assignment of resonances and to the study of structures of proteins and nucleic acids. Distance geometry and molecular dynamics methods (Kaptein et. al., 1985) using NOESY derived inter-proton distances have now been developed that allow us to obtain structural models of short DNA sequences in solution.

We have utilized these methods to analyze **experimentally** the solution structure of a self-complementary DNA octanucleotide, d-GGGTACCC, cross-linked with psoralen. This DNA contains a central 5'-TpA sequence which has been shown to be highly reactive with psoralens. We used 4'-(aminomethyl)-4,5',8-trimethylpsoralen (AMT) to obtain a high yield of cross-link at the central thymines of the oligomer. After assignment of the protons in the sequence with 2D NMR methods, we obtained distance information by peak integration of NOESY experiments. Distance geometry methods were then used to generate models of the cross-linked DNA structure in solution.

Lack of specificity in psoralen intercalation into DNA. We have examined the binding of AMT to the d-GGGTACCC duplex in the absence of light. As has been seen with many intercalators, the addition of AMT leads to broadening and shifting of essentially all aromatic resonances. Both broadening and shift increase in proportion to the amount of drug added and are almost uniform over all bases. These features indicate that the drug binds without significant sequence specificity. The observation of a single resonance for each DNA proton indicates that the drug exchanges among all available binding sites at a rate approaching the fast exchange limit. This is supported by the observed sharpening of resonances with increasing temperature. Similar features are seen in the imino proton region of the spectrum. Furthermore the drug resonances are expected to undergo large upfield shifts upon intercalation and to broaden beyond detection by exchange between available sites and orientations. From the small upfield shifts of the aromatic resonances with increasing drug concentration, and the broadening of linewidth observed, the exchange rate for the drug must be of the order 10^2 sec^{-1}.

Asymmetric destabilization of DNA structure by psoralen cross-linking. In addition to the asymmetry in the structure introduced by the psoralen cross-link, the melting of the DNA detected by NMR is also quite asymmetric. The melting profile of bases, in D_2O, derived from the chemical shifts of resonances as a function of temperature shows clearly that all of the resonances on one side of the DNA douplex begin shifting some 20°C earlier than those on the other side. Similar behavior is also seen for the imino protons. The imino protons on the side which exibits low temperature shifts of aromatic resonances, broaden almost beyond detection, before imino resonances on the opposite side broaden significantly. This cannot come from the intrinsically symmetric DNA sequence and therefore must arise from assymetric perturbation of the DNA structure by psoralen cross-linking.

Psoralen cross-linked DNA is bent and unwound. The bent structure of the AMT cross-linked DNA octamer has been derived from the NMR data. This model was obtained by refining an initial model of the drug-DNA complex in which the DNA was properly base paired, and near the B conformation, to meet the constraints of the distances obtained with NMR. The structure is consistent with the NOE data, with maximum differences between set constraints and distances in the model of<0.3 Å (with only two inter-residue distances violating experimentally determined values by more than 0.2 Å). The photoaddition of the drug significantly disrupts the local structure of the DNA. The protrusion of methyl groups and the amino methyl sidechain from the drug, and the altered geometry of methyls in the photoadducted T's, cause stacking distortion of the adenine bases flanking the cross-link which propagates to the neighboring base pairs through steric interactions. However, excluding the bases immediately flanking the drug, the basic stacking of B form DNA is still observed. The two T residues which are covalently attached to the drug protrude upwards into the neighboring bases significantly. This, together with the methyl groups and side chain on the drug, results in a 53° bend of the DNA double helical axis into the major groove. In addition the DNA is unwound by 56° (assuming an average rotation of 36° between base pairs), and there is a displacement of about 1.5 Å of the helix axes by the covalent addition of the drug. These values are in qualitative agreement with those derived from model building (reported in the last year's report): 45°, 63°, and 1.7 Å for the helix axis kink angle, unwinding angle and helix axis displacement respectively when calculated by the same method of defining helical vectors as here. In this study they are defined by a least squares fitting of the first and last base paired nucleoside of regular duplex to each helical region of the model.

To confirm the bend in the helix we have also carried out full distance geometry calculations on this complex starting with no assumptions about the DNA conformation except the covalent bond structure. These structures show typical distortions of bases and backbone due to the imprecision and incompleteness of constraints in the bounds matrix. However all of these embedded structures refine to give proper base geometries and have approximately the same helix axis bend as the model based structure we present here. The residual error of this model based structure after refinement using the DG or D-space programs is as low as any of the embedded structures.

The distortion of DNA structure proposed provides a structural basis for understanding the results of earlier studies showing changes in DNA sedimentation coefficient, circular dichroism, and thermal denaturation behavior, and in sensitivity to nucleases and chemical reagents. The

angle of bend in the helix axis is also in qualitative agreement with the observations of Griffith et al. (personal communication) using electron microscopy. However our findings are in conflict with those of Sinden and Hagerman (1984), who studied restriction fragments of 80 to 589 base pairs randomly reacted with psoralen, and concluded from gel migration behavior that psoralen cross-linked DNA is not significantly bent. Our melting results suggest significant flexibility of cross-linked DNA, which could explain why bending could not be observed in the longer fragments of randomly modified DNA. Bends have been observed for specific DNA sequences, the most recent of which is the polymerized lambda replication origin. Using uniquely modified polymers of cross-linked DNA would be another way to obtain evidence of bending of the DNA structure.

The bend in the DNA structure may be an important aspect of the perturbation which is recognized by Uvr ABC excision nuclease. This repair enzyme cuts the backbone of the DNA at the -9 and +3 phosphates (with respect to the drug) of the strand containing the furan side adduct. Our model shows that due to the unwinding and the direction of bending of DNA, these two excision sites are on the same side of the bent DNA. Furtermore, the asymmetry in the perturbed structure may be responsible for the ability of this enzyme complex to distinguish the pyrone and furan sides of the adduct. Chemical shift changes show that the perturbation of the DNA structure is minor after 3 bases from the site of the cross-link.

Concluding remarks. We believe that the model presented here correctly describes the structural features of AMT cross-linked DNA. Our data support previous models in which the psoralen cross-linking introduces a significant bend into the DNA helix axis and unwinds the duplex. In addition the NMR data show clearly that AMT cross linking induces a rather strong asymmetry into the structure and the stability of the DNA. Our present model suggests that the structural asymmetry arises primarily from the constraints caused by the formation of the cyclobutane rings between psoralens and thymines on opposing strands, and the steric interactions between substituents on the drug and the neighboring bases. Our hypothesis will be tested by further comparisons with other psoralen cross links. Some of the other covalently damaged DNAs may be similarly distorted and the bent structure may be one of the recognition signals required to initiate DNA repair for these sites. The unwinding of the DNA may also have a long range effect for topologically constrained DNAs, such as circular DNA or DNA in chromatin because the local unwinding will be converted to overall supercoil unwinding. This will affect the DNA packaging of this region and transcription of the genes within the constrained DNA. In the future we plan to do similar studies on other photodamaged DNA structures, including the psoralen monoadduct, and carry out energy refinement and molecular dynamics calculations.

PUBLICATIONS – ENVIRONMENTAL RESEARCH AND DEVELOPMENT

1. *H.B. Gamper, G.D. Cimino, S.T. Isaacs, M. Ferguson, and J.E. Hearst. Reverse Southern Hybridization. Nucleic Acids Research **14**, 9943-9945 (1986).
2. H.B. Gamper, G. Cimino, and J.E. Hearst. Solution Hybridization of Crosslinkable DNA Oligonucleotides to M13DNA: Effect of Secondary Structure on Hybridization Kinetics and Equilibria. J. Mole. Biol., in press (1987).
3. *J.E. Hearst. Background Hybridization Associated with the Use of Photo-Crosslinkable Oligonucleotide Hybridization Probes for the Identification of Unique Sequences in the Human Genome. Photobiochemistry and Photobiophysics, Suppl., 23-32 (1987).
4. S. R. Holbrook, A.H.-J. Wang, A. Rich and S.-H. Kim. Local Mobility of Nucleic Acids as Determined from Crystallographic Data. II. Z-Form DNA. J. Mol. Biol. **187**, 429-440 (1986).
5. A. P. Koretsky, S. Wang, M. P. Klein, T. L. James and M. W. Weiner. P-31 NMR Saturation Transfer Measurements of Phosphorus Exchange Reactions in Rat Heart and Kidney in Situ. Biochemistry **25**, 77-84 (1986).
6. S.E. Lipson and J.E. Hearst. Psoralen Crosslinking of Ribosomal RNA. Methods in Enzymoloby, in press (1987).
7. D. A. Pearlman and S.-H. Kim. Conformational Studies of Nucleic Acids: III. Empirical Multiple Correlation Functions for Nucleic Acid Torsion Angles. J. Biomol. Struct. Dynam. **4**, 049-067 (1986).
8. D. A. Pearlman and S.-H. Kim. Conformational Studies of Nucleic Acids: IV. The Conformational Energetics of Oligonucleotides: d(ApApApA) and ApApapA. J. Biomolec. Struct. Dynam. **4**, 069-098 (1986).
9. T.K. Pratum and M.P. Klein. A ^{14}N and ^{2}H Study of Molecular Motion in Poly crystalline Choline Chloride Salts. J. Chem. Phys.,Springer-Verlag, Berlin, in press.
10. B.A. Scalettar, M.P. Klein, and J.E. Hearst. A Theoretical Study of the Effects of Driven Motion on Rotational Correlations of Biological Systems. Biopolymers **26**, 1287-1299 (1987).
11. *Y. Shi and J.E. Hearst. Thermostability of Double-Stranded Deoxyribonucleic Acids: The Effects of Covalent Additions of a Psoralen. Biochemistry **25**, 5895-5902 (1986).
12. *Y. Shi and J.E. Hearst. The Wavelength Dependence for the Photoreactions of DNA-Psoralen Monoadducts (II): Photocrosslinking of Monoadducts. Biochemistry **26**, 3792-3798 (1987)
13. *H.P. Spielmann, J.D. Kahn, and J.E. Hearst. New Methods for Probing Nucleic Acids. Bioassays **5**, 86 (1986).
14. *T.C. Terwilliger, and S.-H. Kim (1987) Generalized Method of Determining Heavy-Atom Positions using the Difference Patterson Function. Acta Cryst. **A43**, 1-5 (1987)
15. B. Van Houten, H. Gamper, J.E. Hearst, and A. Sancar. Construction of DNA Substrates Modified with Psoralen at a Unique Site and Study of the Action Mechanism of ABC

Exonuclease on these Uniformly Modified Substrates. Journal of Biological Chemistry **261**, 14135-14141 (1986).

16. B. Van Houten, H. Gamper, S.R. Holbrook, J.E. Hearst, and A. Sancar. Action Mechanism of ABC Excision Nuclease on a DNA Substrate Containing a Psoralen Crosslink at a Defined Position. Proc. Nat. Acad. Sci. USA **83**, 8077-8081 (1986).
17. de Vos, A.M. and S.-H. Kim (1987) Crystal Structure of Thaumatin I, a Sweet Taste Receptor Binding Protein. In: Moras, D. et al. (Eds.), **Crystallography in Molecular Biology**, NATO ASI series A, vol. 126, 395-402. Plenum Press: New York.

D. NATIONAL TRITIUM LABELING FACILITY – Professor Henry Rapoport

This laboratory is a national facility both for carrying out research into the labeling of compounds to high specific activity with tritium as well as for providing a tritium labeling service for other investigators throughout the country. A primary direction this laboratory is moving into in the future is the development of compounds and techniques to allow ^3H NMR studies of biological macromolecules.

The establishment of the National Tritium Labeling Facility at LBL, under the sponsorship of the NIH, provides the setting for pursuing exciting new research in structural biology and biophysics. In particular, we propose the use of tritium to expand the molecular weight range of biomolecules that can be studied by Nuclear Magnetic Resonance methods. Amino acids and nucleic acid bases will be selectively tritiated and then specifically incorporated into respective protein and nucleic acid sequences. As the triton is magnetically distinct from the proton and will always be dilute, the spectrum will be simple, the assignment of spectral features straightfoward and the interpretation of the structural and dynamic aspects more site specific than has been possible heretofore. As there is no natural background signal, observation of the ^3H NMR spectra of tritiated substrates, in vivo, provides other opportunities for studying dynamic properties of enzymatics, cellular transport and metabolism.

Summary of Research Progress

In the 05 Grant year, the NTLF has continued to be a resource to the biomedical/biochemical community in the areas of Service, Training, Collaboration and Core Research. The Facility has been greatly enhanced over the last year by the installation of the 300 MHz ^3H NMR and our very recent acquisition of a Biosearch 8600 DNA synthesizer (made available to us through the Department of Chemical Biodynamics). These instruments, coupled with our high level labeling capability, make the NTLF an unique laboratory which, is attracting the attention of many potential new users and collaborators. In addition the Facility has been improved with the addition of a portable building which provided much needed office space for the staff and users, separate from the laboratory facilities. We have also added a new scintillation counter and are about to test a tritium stack monitor designed and assembled at the NTLF.

Service and Training

During the first 9 months of the current budget year the NTLF has assisted 17 users from 11 institutions, both academic and industrial, in labeling 30 different compounds . Over half of these users were new to the NTLF and all received training in the handling and manipulation of high specific activity tritiated compounds while they were here. The NTLF also supported the work of two graduate students and one undergraduate student (summer).

Collaborative Research

The NTLF has placed special emphasis on collaborative research this past budget year. At the present time we are involved in six projects, three of which involve outside organizations (Stanford Magnetic Resonance Lab, VA Medical Center Dermatology Dept., San Francisco, and the Cancer Center, University of Texas). These projects cover a wide range of interests, including the synthesis of labeled proteins and peptides (Tuftsin), ^{3}H NMR studies of maltose binding protein and antibody F_{ab} fragments and the synthesis of labeled free radical traps, all of which should be of great interest to the biomedical/biochemical community. Although these projects have only just been started, there are already some interesting results from the maltose binding protein study.

Core Research

Core research has continued in three areas; synthetic labeling methods, tritium NMR spectroscopy and excitation labeling. The overall direction of our core research in these three areas is to develop methods to label molecules of current biological interest (small proteins, peptides and their precursors) and to study reaction mechanisms, binding and structures using these labeling techniques in conjunction with the ^{3}H NMR.

1. **Synthetic Labeling** Projects for this year included the synthesis and selective labeling of putrescine dihydrochloride, novel syntheses of high specific activity monotritiated methyl iodide and multihalogenated precursors for the generation of tritritiated methyl iodide, studies of heterogeneous group VIII metal-catalyzed exchange, solvent exchange, synthesis of labeled DNA oligomers and the synthesis of tritium labeled food mutagens. These projects represent important synthetic contributions to biochemical/biomedical research which can be used in academia and industry. In particular the methyl iodide, metal-catalyzed exchange, solvent exchange and DNA oligomer synthesis will be of wide general utility and interest.

2. **Tritium NMR Spectroscopy** Projects for this year included studies of tritium NMR techniques (correlation spectroscopy, multiple quantum spectroscopy, polarization transfer and indirect detection methods), calculation of the sensitivity of tritium NMR in terms of molarity and radioactivity, the preliminary design of probes for performing tritium NMR on solids or semi-solids and the application of tritium NMR to the study of glycolysis in blood. Observation by ^{3}H NMR of the conversion of glucose, labeled at the C-1 position, to lactate by erythrocytes indicated that the two anomers of glucose disappear at different rates and that the rate constant for the disappearance of the (alpha) anomer and for the appearance of lactate are equal and also equal to the anomeric interconversion rate. The result of the study of the sensitivity of tritium NMR indicates that the single pulse sensitivity to 30 mCi of tritium (1 micromole) is 140:1 which agrees with the theoretical calculation and means that we can probably get useful information at levels below 1 mCi.

These projects demonstrate the utility of using tritium NMR for biological and physiological

research and provide some of the tools for carrying out this work. The glycolysis project has been of particular interest because it is a demonstration of how the metabolic reactions of an important substrate can be followed using a non-invasive technique.

3. **Excitation Labeling** Projects for this year included studies of microwave discharge activated (MDA) exchange into simple organic molecules, studies of MDA peptide labeling, studies of MDA labeling using tritium adsorbed on supported metal catalysts and studies of thermally activated tritium exchange. As indicated by radio-GLC and ^3H NMR analysis, both MDA and thermal activation have one major drawback; they both cause aromatic ring saturation. We have found, however, that peptides containing no phenylalanine or tyrosine residues were labeled with high radiochemical yield and that N,N-dimethylaniline was not saturated using MDA.

Excitation labeling is a particularly attractive method of labeling for many of our users and collaborators. We will continue research in this area with the hope of providing a general labeling method that can be used for materials that are difficult or impossible to do any other way.

Highlights

The following two subprojects illustrate contributions by the National Tritium Labeling Facility in the area of labeled synthesis. The reagent for the preparation of tritiated methyl iodide will be of great value for N-tritiomethylation reactions.

1. Synthesis and Selective ^3H Labeling of Putrescine Dihydrochloride

A series of putrescine dihydrochlorides specifically labeled at the 2 and 3 positions has been prepared by hydrogenation of olefinic and acetylenic precursors with deuterium or tritium gas. The olefinic precursor was synthesized by a modification of the Delpine reaction. The acetylenic compound was prepared in a new, high-yielding (85%), chemoselective manner. Hydrogenation of the dihydrochloride salts of the precursors was conducted under an atmosphere of T_2 or D_2 gas, in the presence of Pd-C catalyst, with DMSO as solvent. Features of this experimental design were that labeling was the final synthetic step, and that removal of the solvent after hydrogenation yielded a pure, crystalline product. ^1H, ^2H and ^3H NMR spectra of the products were recorded immediately after their labeling, and the level of incorporation and positions of the stable and radioactive hydrogens were determined.

This project is now complete, and a manuscript entitled "Specific Labelling of Putrescine Dihydrochloride by Heterogeneous Hydrogenation with Deuterium or Tritium Gas in Dimethyl Sulfoxide" has been submitted for publication in the Journal of Organic Chemistry (April 28, 1987).

2. A Novel Synthesis of Monotritiated Methyl Iodide – A Regent for [^{3}H]-N-Methylation and Similar Reactions

A new and efficient synthesis of monotritiated methyl iodide was achieved by preparing the chloromethyl and bromomethylbenzoates and chloromethyl 4-phenylbenzoate. Tritiodehalogenation of the esters gave the monotritiomethyl esters with radiochemical purity of better than 98%. Cleavage of the esters by lithium iodide at 140-150*deg*C afforded [^{3}H]-methyl iodide in high yield. The last reaction can be performed neat in the case of liquid esters and with an aprotic solvent (DMF) with solid esters. Vacuum transfer of the tritium labeled methyl iodide into a solution of N,N-dimethylaniline afforded [^{3}H]-trimethylphenyl ammonium iodide in 74% yield.

Monotritiomonodeuteromethyl 4-phenylbenzoate was also synthesized as the precursor for the preparation of (dl) chiral methyl iodide. The specificity of the labeling in the final products was then confirmed by ^{3}H NMR.

The following two subprojects are examples of the contribution by the National Tritium Labeling Facility in the area of ^{3}H NMR spectroscopy. The techniques of *in vitro* monitoring used in these studies should be of great interest and value in biochemical/biomedical research.

3. Application of Tritium NMR to the Study of Glycolysis in Blood

The conversion of glucose (labeled at the C-1 position) to lactate by erythrocytes was monitored by ^{3}H NMR. The rate constants for the two anomers of glucose, the appearance and disappearance of one intermediate and for the appearance of lactic acid were determined. The surprising result of the study is that the two anomers disappear at different rates and that the rate constant for the disappearance of the alpha anomer and for the appearance of lactate are equal and also equal to the anomeric interconversion rate.

4. Interaction of Maltose with Maltose Binding Protein

Studies have begun on the effect that the binding of maltose has on the solution structure of maltose binding protein (MBP). ^{1}H NMR experiments are limited by the huge number of lines in the NMR spectrum (even at 500 MHz) and attempts to characterize the binding site have been limited. In order to characterize the kinetic parameters of maltose binding, to evaluate the relative binding of the alpha and beta anomers and to try to characterize the groups on the protein which are in contact with the sugar, maltose was specifically labeled in the C-1 position by catalytic exchange. ^{3}H NMR indicated an extremely clean product.

In preliminary binding studies, approximately equimolar concentrations of tritiated maltose and the binding protein have been studied by both ^{3}H and ^{1}H NMR. Addition of a molar excess of maltose leads to ^{3}H signals for both the free and bound maltose, as expected. It was interesting to see that the ratio of signals seen for the bound alpha and beta forms is not the same as for the free excess maltose present with the protein, the difference being about 25%. While it is not surprising that there is some difference in the binding constant for the two forms, this difference

is very difficult to determine using other physical methods.

Initial attempts at heteronuclear NOE experiments to try to characterize the protein binding site further suggest that the maltose is bound close to a methionine methyl group.

The following subproject is an example of the contribution of the National Tritium Labeling Facility in the area of excitation labeling.

5. Microwave Discharge Activated Peptide Labeling

A number of di- and tripeptides were dispersed on catalytic surfaces and labeled with trtium. The labeled products were analyzed by HPLC. Phenylalanine was labeled on different catalysts such as Ru on silica-alumina, alumina, Pd and vanadium catalysts, and silica-alumina in order to determine the influence of the dispersion medium on the radiochemical yield and the extent of ring saturation. Among the catalysts, Ru gave the highest radiochemical yields, but the vanadium catalyst appeared to be a better exchange catalyst. The labeled products consisted mainly of the saturated product, cyclohexylalanine.

Peptides containing no phenylalanine or tyrosine residues were labeled with high radiochemical yield, but those containing phenylalanine or tyrosine residues formed mainly saturated analogs. If the ring saturation reaction can be avoided by the manipulation of the catalyst and conditions or by employing an alternative method such as thermal activation, we will have a very useful method for peptide labeling.

Resource Advisory Committee and Allocation of Resources

One undergraduate received training at the facility.

For this budget year the NTLF Advisory Committee consisted of:

Name	Department	Institution
Thomas Budginger	Biomedical	LBL, UC Berkeley
Nicholas Matwiyoff	Center for Non-Invasive Diagnosis	Los Alamos NL, NM
Kenneth Rinehart	Chemistry	Univ. of Illinois
Alexander Susán	Radiochemistry	Sandoz,Inc.
Leonard Spicer	Analytical NMR Spec-troscopy Center	Duke University, NC
William Trager	Medicinal	U. of Washington

The annual meeting was held at the NTLF on February 28, 1987.

Objectives for the Coming Year

The following are the objectives for the NTLF for the 06 Grant year:

1. Maintain service activities at the current level of 10-20 users per year, but expand our base of users through publicity.

2. Increase the number of collaborative projects with outside groups (academic and industrial). These longer term projects are advantageous both to the NTLF and the outside groups with respect to the exchange of information and techniques. We are currently involved in 6 collaborative projects and would like to expand this area as much as possible.

3. Focus on developing methods to label small proteins, peptides and their precursors and to study reaction mechanisms, binding and structures using these labeling techniques in conjunction with 3H NMR spectroscopy. This work will be carried on through our core research and collaborative programs.

4. Continue studies on general 3H NMR techniques, heterogeneous and homogeneous exchange reactions and solvent exchange under catalytic hydrogenation conditions.

5. Continue studies in excitation labeling techniques (microwave and thermal) concentrating on obtaining a useful method for tritiation of peptides and proteins.

PUBLICATIONS – NATIONAL TRITIUM LABORATORY FACILITY

1. Richard E. Moore, Gregory M.L. Patterson, Michael Entzeroth, Hiromi Morimoto, Masami Suganuma, Hiromi Hakii, Hirota Fujiki, and Tukashi Sugimura. Binding Studies of I[^{3}]Hlyngbyatoxin A and [^{3}H]debromaplysiatozin to the Phorbol Ester Receptor in a Mouse Epidermal Particulate Fraction. Carcinogenesis [7], no 4., pp. 641-644, 1986.
2. R. L. Hua and Chin T. Peng. Relative Efficiencies of Tritium Atoms and Ionic Species in Peptide Labeling, J. Labelled Compd. Radiopharm. **24**, in press (1987).
3. C. T. Peng. Tritium Labeling Using Adsorbed Tritium and Catalytic Surfaces. In **Synthesis and Applications of Isotopically Labeled Compounds** 1985, (R.R. Mucinno, Ed), Elsevier Science Publ. B.V., Amsterdam, 1986, pp. 155-154.
4. C. T. Peng. Radiation Induced Methods of Labeling. In **Isotopes in Physical and Biomedical Sciences**, (J.R. Jones and E. Buncel, Eds), Elsevier, Amsterdam, 1987, Chapter 2, 46 pp.
5. C. T., Peng and Ruoling L. Hua. Comments on Tritium Monitoring by Liquid Scintillation Counting, Appl. Radiat. Isot. **38**, in press (1987).

E. DOE SPONSORED UNIVERSITY CONTRACT – Professor Ignacio Tinoco, Jr.

Left-Handed RNA

Left-handed Z-RNA was discovered in our laboratory in 1984; it was the first new double-helical structure found for RNA since right-handed A-RNA was described in the 1950s. The Z-RNA conformation occurred when a polyribonucleotide with a sequence of alternating cytosines and guanines [poly r(C-G)] was placed in highly concentrated aqueous solutions of salts (6 M NaBr or $NaClO_4$). The most important questions to ask about a new structure for a biological molecule are: (1) Does the structure exist in nature? (2) Does it have a biological function? We have made antibodies against left-handed Z-RNA to provide answers to these questions. The anitbodies were made by injecting brominated poly r(C-G) into rabbits; the bromination favors the Z-conformation even in physiological salt concentrations. Two classes of antibodies were obtained; one class reacts both with left-handed Z-RNA and Z-DNA, but the other class only reacts with Z-RNA. The high specificity of antibodies provides a tool to search complex biological mixtures for left-handed Z-RNA structures. In collaboration with Dr. David Zarling of SRI International in Menlo Park we have found that the anti-Z-RNA antibody specifically binds in the cytoplasm of fixed protozoan cells. Pre-treatment of the cells with ribonuclease, an enzyme which destroys RNA, prevents the binding; this validates that the binding is to RNA. The next step is to identify what RNA molecules are involved. Once the left-handed molecules are located, the possible biological functions of these unusual entities can be investigated.

Differential Polarizing Microscope

A microscope which produces images dependent only on linear dichroism or on ciruclar dichroism of the object has been developed. This provides sensitive new ways of looking at cells which do not require fixation and staining. Two biological systems are being studied with this microscope: erythocytes from patients with sickle cell disease and spermatocytes from Drosophila.

The polymerization of hemoglobin and the orientation of hemoglobin polymer can be visualized directly in single sickle cell erythrocytes. The influence of oxygen pressure and the rate of change of oxygen pressure has been studied. Previous microscopic studies of single cells have been limited to the effect of oxygen on overall cell shape. Our new studies provide information on both cell shape and hemoglobin orientation. This correlation between the molecular damage in sickle cell disease (a mutation in the hemoglobin molecule which favors polymerization) and the effect on cellular distortion may make it easier to learn how to control the deleterious effects of the disease.

An investigation of changes in cellular organization during spermatogenesis in Drosophila has been started. Unfixed and unstained cells in different stages of development have been observed using linear and cirular dichroism. Characteristic changes are visible in the nucleus and the nucleolus which correlate with the degree of synthetic activity taking place in the cells at that time.

Base Mismatches and Mutagenesis

Study of the thermodynamics and structures of DNA double helices containing base-base mismatches (non-Watson-Crick base pairs) is continuing in this laboratory. Adenine-adenine and thymine-thymine mismatches did not disrupt the DNA duplex greatly. Nuclear magnetic resonance measurements showed that the helix base pairs on either side of the mismatch were not very different from those in normal duplexes. We are trying to correlate the thermodynamics of formation of mismatches to the probability of mutation.

PUBLICATIONS – DOE SPONSORED UNIVERSITY CONTRACT

1. F.H. Arnold, S. Wolk, P. Cruz and I. Tinoco, Jr. Structure, Dynamics and Thermodynamics of Mismatched DNA Oligonucleotide Duplexes $d(CCCAGGG)_2$ and $d(CCCTGGG)_2$. Biochemistry **26**, 4068-4075 (1987).
2. P. Cruz, K. Hall, J. Puglisi, P. Davis, C.C. Hardin, M.O. Trulson, R.A. Mathies, I. Tinoco, Jr., W.C. Johnson, Jr., T. Neilson. The Left-Handed Z-form of Double-Stranded RNA. In: **Biomolecular Stereodynamics, IV**, Adenine Press, New York, pp. 179-200, 1986.
3. P.W. Davis, K. Hall, P. Cruz, I. Tinoco, Jr., and T. Neilson. The Tetraribonucleotide rCpGpCpG Forms a Left-Handed Z-RNA Double Helix. Nucleic Acids Research **14**, 1279-1291 (1986).
4. C.C. Hardin, D.A. Zarling, J.D. Puglisi, M.O. Trulson, P.D. Davis, and I. Tinoco, Jr. Stabilization of Z-RNA by Chemical Bromination and its Recognition by Anti-Z-DNA Antibodies. Biochemistry, in press, (1987)
5. M.F. Maestre and I. Tinoco, Jr. Differential Imaging Device, U.S. Patent Appliction. 1986.
6. W.E. Mickols, J.D. Corbett, M.F. Maestre, I. Tinoco, Jr., J. Kropp, S.H. Embury. The Effect of Speed of Deoxygenation on the Percentage of Aligned Hemoglobin in Sickle Cells. Application of Differential Polarization Microscopy. J. Biol. Chem., in press.
7. W.E. Mickols, M.F. Maestre and I. Tinoco, Jr. Differential Polarization Microscopy of Changes in Structure in Spermatocyte Nuclei. Nature, in press.
8. C.L. Phillips, W. Mickols, M.F. Maestre and I. Tinoco, Jr. Circular Differential Scattering and Circular Differential Absorption of DNA-Protein Condensates and of Dyes Bound to DNA-Protein Condensates. Biochemistry **25**, 7803-7811 (1986).
9. I. Tinoco, Jr., P. Cruz, P. Davis, K. Hall, C.C. Hardin, R.A. Mathies, J. D. Puglisi, M.O. Trulson, W.C. Johnson and T. Neilson. Z-RNA: A Left-Handed Double Helix. In: **Structure and Dynamics of RNA**, Plenum Press, New York, 1986.
10. I. Tinoco, Jr. This Week's Citation Classic. Current Contents Life Sciences **17**, 16 (1986) and Current Contents Engineering, Technology and Applied Sciences **17**, 16 (1986).
11. I. Tinoco, Jr., W. Mickols, M. Maestre, and C. Bustamante. Absorption, Scattering and Imaging of Biomolecular Structures with Polarized Light. Ann. Rev. Biophysics Biophys. Chem. **16**, 319-349 (1987).

12. I. Tinoco, Jr., S. Wolk, F. Arnold, F. Aboul-ela. Structure and Function in Nucleic Acids: Mutagenesis. In **Structure and Dynamics of Biopolymers**, C. Nicolini, Ed, Plenum Press, in press (1987).

13. A. L. Williams, Jr., C. Cheong, I. Tinoco, Jr. and L. B. Clark. Vacuum Ultraviolet Circular Dichroism as an Indicator of Helical Handedness in Nucleic Acids. Nucleic Acids Research **14**, 6649-6659 (1986).

14. A.L. Williams, Jr. and I Tinoco, Jr. A Dynamic Programming Algorithm for Finding Alternate RNA Secondary Structures. Nucleic Acids Research **14**, 299-315 (1986).

IV. APPENDICES

A. CHEMICAL BIODYNAMICS DIVISION THESES

1986

1. Paul J. Carson. M.S. Thesis. Application of Tritium NMR to the Study of Glycolysis in Blood. December 1986. (Melvin P. Klein). Biophysics.

1987

1. Grassian, Vicki. Ph.D. Thesis. Structural Analysis and Photochemistry of Adsorbates on Platinum and Palladium Surfaces. April 1987. (Professor George C. Pimentel). Chemistry.
2. Scalletar, Bethe Anne. Ph.D. Thesis. Fluroescence Spectrosopic Studies in DNA Dynamics. April 1987. (John E. Hearst and Melvin P. Klein, Co-Charimen) Biophysics.
3. Switalski, Stephen Casimir. Picosecond Spectroscopy Studies of Energy Transfer in Phycobiliproteins and Model Dye Systems. February 1987. (Professor Kenneth Sauer). Chemistry.

B. STATISTICAL DATA

1. Postdoctoral Visitors, Chemical Biodynamics Division July 1, 1986 through June 30, 1987

	Name	Source of Funding	Associate
	Ansari, Mohdaslam	UC	Henry Rapoport
	Braathen, Gier	Norwegian Research Council	George Pimente
	Brewer, Karen	LBL	Melvin Calvin
	Chou, Pi-Tai	LBL	Heinz Frei
*	Consani, Keith	LBL	George Pimentel
	DeVos, Abraham	UC	Sung-Hou Kim
*	Fiege, Ursula	LBL	Kenneth Sauer
	Gingrich, Jeffrey	LBL	Kenneth Sauer
*	Goswami, Kishaloy	LBL	Melvin Calvin
	Grassian, Vicki	UC	George Pimentel
	Holbrook, Libby	UC	Sung-Hou Kim
	Hong, Yong-Ki	Korean Science Engineering Foundation	Sung-Hou Kim
	Kallick, Deborah	UC	David Wemmer
	Keuper, Herman	LBL	Kenneth Sauer
	Konwer, Dolan	Govt. of Assam, India	Melvin Calvin
*	Leone, Anthony	LBL	Melvin Calvin
*	Li, Zong Qi	UC	Sung-Hou Kim
*	Maliyackel, Anthony	LBL	Melvin Calvin
	Mattias, Pedro	UC	Sung-Hou Kim
*	Mickols, William	UC	Ignacio Tinoco, Jr.
	Orton, Edward	LBL	George Pimentel
	Salama, Farid	LBL	Heinz Frei
	Scalletar, Bethe	UC	Melvin Klein
	Thurmes, William	UC	Ignacio Tinoco, Jr.
	Walker, George	UC	Ignacio Tinoco, Jr.
	Yachandra, Vittal	UC	Melvin Klein
	Zimmerman, Jean-Luc	CEN Saclay, DB/SGPh, France	Melvin Klein

* Terminated

STATISTICAL DATA - Cont.

2. Graduate Students, Chemical Biodynamics Division July 1, 1986 to June 30, 1987

Name	Department	Research Director
* Sam Abrash	Chemistry	George Pimentel
* Douglas Anthon	Chemistry	John Clark
Gregory Armstrong	Chemistry	John Hearst
Enoch Baldwin	Chemistry	John Hearst
John Bishop	Chemistry	Henry Rapoport
David Britt	Physics	Melvin Klein
* Ward Brown	Chemistry	George Pimentel
Donald Burke	Chemistry	Hearst
Ann Caviani	Chemistry	David Wemmer
Chae Joon Cheong	Chemistry	Ignacio Tinoco, Jr.
Edgar Civitello	Chemistry	Henry Rapoport
Andrea Cochran	Chemistry	Peter Schultz
James Cole	Chemistry	Kenneth Sauer
James Corbett	Chemistry	Ignacio Tinoco
David Cook	Chemistry	John Hearst
Victoria DeRose	Chemistry	Kenneth Sauer
Susan Dexheimer	Physics	Melvin Klein
Marc Donsky	Chemistry	Henry Rapoport
Paul Gordon	Biophysics	Sung-Hou Kim
Ronald Guiles	Chemistry	Melvin Klein
Douglas Hawkins	Chemistry	Melvin Calvin
Carolyn Hoener	Chemistry	George Pimentel
* Ya-Ming Hou	Comparative Biochemistry	Sung-Hou Kim
Michael Howard	Chemistry	Henry Rapoport
John Hubbard	Chemistry	John Hearst
Nathan Hunt	Physics	Melvin Klein
Fan Jiang	Biophysics	Sung-Hou Kim
David Jones	Chemistry	Henry Rapoport
Chul Hee Kang	Biophysics	Sung-Hou Kim
Gary Karp	Chemistry	Henry Rapoport
* Hiromi Komiya	Comparative Biochemistry	Sung-Hou Kim
Sandra Laursen	Chemistry	George Pimentel
William Lubell	Chemistry	Henry Rapoport
Michael Matsko	Genetics	Sung-Hou Kim
Catherina Maulbecker	Comparative Biochemistry	James Bartholomew
Michael Milburn	Chemistry	Sung-Hou Kim

	Patricia Maxson	Chemistry	Kenneth Sauer
	Ann McDermott	Chemistry	Kenneth Sauer
	Joseph Montforte	Chemistry	John Hearst
	Meredith Morgan	Chemistry	George Pimentel
	Ishita Mukerji	Chemistry	Kenneth Sauer
*	Roberta Mulford	Chemistry	George Pimentel
	Warren Niles	Physics	Melvin Klein
	David O'Brien	Chemistry	John Hearst
	John O'Connell	Chemistry	Henry Rapoport
*	Craig Ogata	Chemistry	Sung-Hou Kim
	Joseph Pease	Chemistry	David Wemmer
	Jeffrey Pelton	Chemistry	David Wemmer
	Joseph Puglisi	Chemistry	Ignacio Tinoco
	Nick Pugliano	Chemisry	George Pimentel
	Jognan Rhee	Chemistry	Kenneth Sauer
	Renee Roemmele	Chemistry	Henry Rapoport
*	Bethe Scalettar	Biophysics	Melvin Klein
	William Scott	Chemistry	Sung-Hou Kim
	Paul Selvin	Physics	Melvin Klein
*	Cynthia Skrukrud	Comparative Biochemistry	Melvin Calvin
	Elizabeth Snowdon	Chemistry	David Wemmer
	Richard Storrs	Chemistry	Kenneth Sauer
*	Sarah Tabbut	Chemistry	Kenneth Sauer
	Liang Tong	Chemistry	Sung-Hou Kim
	Thein V. Truong	Chemistry	Henry Rapoport
	Andrew Van Sickle	Chemistry	Henry Rapoport
	Sun Un	Chemistry	Melvin Klein
	Milton Werner	Chemistry	David Wemmer
	Brent Wurfel	Chemistry	George Pimentel
	Muh-Ching Yee	Chemistry	James Bartholomew

* Terminated

STATISTICAL DATA - Cont.

3. Graduate Student Statistics : Chemical Biodynamics Division 1948–1987

Departments/(Groups) of the University represented (Ph.D. Theses):

	1986–1987	Total to June 30, 1987
Chemistry	2	135
Biophysics	1	17
Compartive Biochemistry	1	10
Physics		7
Plant Physiology		4
Psychology		3
Biochemistry		3
Molecular Biology		3
Agricultural Chemistry		2
Botany		2
Geology		1
Physiology		1
Mathematics		1

189 Ph.D. degrees awarded 1948–1987

11 M.S. degrees awarded 1948–1987

STATISTICAL DATA - Cont.

4. Chemical Biodynamics Division Staff July 1, 1986 – June 31, 1987

Faculty Associates (Chemistry Department)
George C. Pimentel, Director
Melvin Calvin
John E. Hearst,Acting Director
Sung-Hou Kim
Henry Rapoport
Kenneth Sauer
Ignacio Tinoco, Jr.
David Wemmer

Senior Scientific Staff

James C. Bartholomew
Melvin P. Klein
John W. Otvos

Divisional Fellows
Heinz Frei
Francesca Leach

Administrative Staff
Lois Soule, Administrator
Mary Bundy
Gloria Goldberg
Beth Klingel
Rosalyn Miles
Evangeline Peterson
Marilyn Taylor

Scientific and Technical staff
Marie Alberti
Matt Bann
Duncan Beniston
Al Dorsky
Phil Eggers
Benjamin Gordon*
Everett Harvey
Libby Holbrook
Stephen Holbrook
Jarmila Jancarik
Rosalind Kim
David Koh
Ethel Lefall
Marian Malone
Hiromi Morimoto
Ann Orme
Gary Smith
Manouchar Saljoughian
Janet Splitter
Scott Taylor
Philip Williams
Hisao Yokota
Yu-Sheng Zhu

Undergraduates
Paul Carson
Patty Casey
Johnson Chan
John Kim
Jun Kim
Hank Lee
Chris Orme
Hans Peterson
Catherine Swan

Graduate Students, Postdoctoral Visitors and Faculty listed separately.
* terminated.

5. Visiting Faculty July 1,1986 – June 30, 1987

* John Allen
Dept. Plant Sciences
University of Leeds
(Kenneth Sauer)

George A. Brooks
Department of Physical Education
University of California, Berkeley
(James Bartholomew)

Alexander Glazer
Bacteriology and Immunology Dept.
University of California, Berkeley
(Kenneth Sauer)

* Alfred Holzwarth
Max-Planck Institute fur Strahenchemie,
Mulheim, West Germany
(Kenneth Sauer)

Yoichi Namariyama
Kyushu Sangyo University
Fukuoka, Japan
(Melvin Calvin)

Ning Pon
Biochemistry Department
University of California, Riverside
(Henry Rapoport)

* Rafael Rafaeloff
Dept. of Pure and Applied Radiation Chemistry
Soreq Nuclear Research Center, Ravine, Israel
(Melvin Calvin)

Michael Saxton
Plant Growth Laboratory
University of California, Davis
(Melvin Klein)

Lawrence Spreer
Department of Chemistry
University of Pacific, Stockton
(John Otvos)

TECHNICAL INFORMATION DEPARTMENT
LAWRENCE BERKELEY LABORATORY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIFORNIA 94720